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(54) **Title:** ADENOVIRUS PARTICLES WITH MUTAGENIZED FIBER PROTEINS

(57) **Abstract:** This invention relates to mutated adenoviral fiber proteins and adenovirus particles containing such proteins. It further relates to polynucleotides encoding the proteins and vectors containing polynucleotides. It also relates to methods for making and using the adenoviral particles. With the mutated fiber proteins, the adenovirus particles no longer bind to their natural cellular receptor. They can then be "retargeted" to a specific cell type through the addition of a ligand to the virus capsid, which causes the virus to bind to and infect such cell. Specific fiber mutations are listed, which ablate binding to the natural receptor. Adenovirus particles with certain fiber mutations were found to enhance gene transfer to and expression in liver as compared to viral particles with wild-type fiber.

ADENOVIRUS PARTICLES WITH MUTAGENIZED FIBER PROTEINS

BACKGROUND OF THE INVENTION

The wide tropism of adenoviral vectors is one of its advantages as a gene-delivery vehicle. However, there are a number of reasons why targeted vectors are desirable. Adenoviral vectors with increased transduction specificity should show reduced toxicity, since lower doses could be delivered to achieve the same desired therapeutic benefit. In addition, these lower doses should reduce potential immune responses to the viruses. This increased safety of targeted vectors would then allow for new routes of delivery, such as systemic administration, that would be applicable to a number of indications, like cancer and cardiovascular disease. Adenoviral particles with mutagenized fiber proteins are useful in the preparation of targeted adenoviruses.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the strategy used for the production of pseudotyped adenoviral vectors with transiently expressed fiber proteins using the transient transfection/infection system. Figure 1A shows a schematic diagram of the genomic structure of Ad5.βgal.ΔF. Figure 1B shows the transient transfection/infection system. The fiber deleted adenoviral vector, Ad5.βgal.ΔF, as shown in panel A, can be grown in packaging cell lines transiently or stably expressing different fiber proteins to generate Ad5.βgal.ΔF/F+ fiber containing adenoviral particles. The vector is used to infect 293T cells that have been transfected with a fiber expression plasmid. The resulting particles will have new receptor tropisms dependent on the fiber protein.

Figure 2 shows the differential fiber-dependent adenoviral transduction properties of HeLa cells using pseudotyped adenoviral vectors. HeLa cells were transduced with 1000 total particles per cell with the indicated pseudotyped adenoviral vector. After 24 hours, the cells were analyzed for β-galactosidase activity using a chemiluminescence reporter assay. The relative β-galactosidase activity of each pseudotyped adenoviral vector containing a mutated fiber protein was determined and normalized as a percentage of Ad5.βgal.ΔF/wt, which contains a wildtype fiber protein. All values are the mean percentage of Ad5.βgal.ΔF/wt, ± standard deviation (sd) derived from 5 to 6 separate transductions.

Figure 3 is a plasmid map for p5FloxPRGD.

Figure 4 is a plasmid map for pAv1hlpr.

Figure 5 is a plasmid map for pSKO2, containing fiber mutations in combination with a cRGD targeting moiety.

Figure 6 shows the transduction efficiency of adenovirus with retargeting ligand and detargeting fiber mutations. HDF (Fig. 6A), HeLa (Fig. 6B), CHO-K1 (Fig. 6C), and PC3 (Fig. 6D) cells were infected at 20 to 12500 total particles per cell in five-fold dose increments with the indicated fiber-modified adenoviral vectors. Av1nBg is the parental control with an unmodified fiber gene, Av1nBgHIRGD has been genetically altered to include cRGD in the HI loop, Av1nBgHIRGDKO1 has been genetically altered to include cRGD in the HI loop and the S408E, P409A mutation in fiber knob, and Av1nBgHIRGDKO2 has been genetically altered to include cRGD in the HI loop and the ΔV441, K442 mutation in fiber knob. After 24 hours, the cells were analyzed for ß-galactosidase reporter gene activity using a chemiluminescence reporter assay.

Figure 7 shows that Av1nBgHIRGDKO2 can compete transduction of HDF cells with Av1GFPHIRGD. HDF cells were infected at 1000 particles per cell with Av1GFPHIRGD, an adenoviral vector expressing GFP and which has been genetically altered to include cRGD in the HI loop. The infections were competed with Av1nBg, Av1nBgHIRGD, Av1nBgHIRGDKO1, and Av1nBgHIRGDKO2 at doses ranging from 1000 to 128,000 particles per cell in four-fold dose increments. After 24 hours, the cells were analyzed for GFP expression by measuring the fraction of cells that were positive for GFP expression by FACS analysis. The data was normalized as a percentage of Av1GFPHIRGD without competitor.

Figure 8 is a plasmid map for pSKO1, containing fiber mutations in combination with a cRGD targeting moiety.

Figure 9 is a plasmid map of pFLAv3nBgKO1 containing the full-length adenoviral genome with the KO1 fiber AB loop mutation.

Figure 10 shows the transduction efficiency of Hela (Fig. 10A) and HDF (Fig. 10B) cells using adenoviral vectors containing fiber AB loop mutations.

Figure 11 shows the transduction efficiency of Hep3B (Fig. 11A), HepG2 (Fig. 11B) and mouse hepatocytes (Fig. 11C) using adenoviral vectors containing fiber AB loop mutations.

Figure 12 shows a competition viral transduction assay.

Figure 13 shows *in vivo* adenoviral-mediated expression of β-galactosidase by an analysis of β-galactosidase activity in mouse livers.

Figure 14 shows *in vivo* adenoviral-mediated transduction of mouse livers by hexon PCR analysis.

Figure 15 shows *in vivo* adenoviral-mediated expression expression of β-galactosidase by an analysis of β-galactosidase activity in C57BL/6, Balb/C, and CD-1 mouse livers.

Figure 16 shows *in vitro* adenoviral-mediated transduction of isolated primary CD-1 mouse hepatocytes.

DESCRIPTION OF THE INVENTION

This invention relates to mutated adenoviral fiber proteins and adenovirus particles containing such proteins. It further relates to polynucleotides encoding the proteins and vectors containing the polynucleotides. It also relates to methods for making and using the adenoviral particles. With the mutated fiber proteins, the adenovirus particles no longer bind to their natural cellular receptor. They can then be "retargeted" to a specific cell type through the addition of a ligand to the virus capsid, which causes the virus to bind to and infect such cell.

As used herein, the term "adenovirus" or "adenoviral particle" is used to include any and all viruses that may be categorized as an adenovirus, including any adenovirus that infects a human or an animal, including all groups, subgroups, and serotypes. Preferably, such adenoviruses are ones that infect human cells. Such adenoviruses may be wild-type or may be modified in various ways known in the art or as disclosed herein. Such modifications include modifications to the adenovirus genome that is packaged in the particle in order to make an infectious virus. Such modifications include deletions known in the art, such as deletions in one or more of the E1, E2a, E2b, E3, or E4 coding regions. Such modifications also include deletions of all of the coding regions of the adenoviral genome. Such adenoviruses are known as "gutless" adenoviruses. The terms also include replication-conditional adenoviruses; that is, viruses that replicate in certain types of cells or tissues but not in other types. These include the viruses disclosed in U.S. Patent No. 5,998,205, issued December 7, 1999 to Hallenbeck et al. and U.S. Patent No. 5,801,029, issued September 1, 1998 to McCormick, the disclosures of both of which are incorporated herein by reference in their entirety. Such viruses are sometimes referred to as cytolytic or cytopathic viruses (or

vectors), and, if they have such an effect on neoplastic cells, are referred to as oncolytic viruses (or vectors).

In one embodiment, the mutated adenoviral fiber protein of the invention is a fiber protein where at least one amino acid in the CD loop of a wild-type fiber protein of an adenovirus from subgroup C, subgroup D, subgroup E, or selected viruses from subgroup F, (in particular those having the long fiber) have been mutated to reduce or substantially eliminate the ability of the fiber protein to bind to the cellular receptor known as the coxsackievirus-adenovirus receptor (CAR) to which the wild-type fiber of these subgroups, as well as subgroup A, bind. These subgroups are standard taxonomic designations known to those skilled in the art. Subgroup A includes adenovirus serotypes 12, 18, and 31. Subgroup C includes adenovirus serotypes 1, 2, 5, and 6. Subgroup D includes adenovirus serotype 8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, and 42-49. Subgroup E includes adenovirus serotype 4. Subgroup F includes adenovirus serotypes 40 and 41. These latter two serotypes have both a long and a short fiber protein. Only the long fiber protein binds to CAR. The preferred adenovirus serotype of the invention is adenovirus serotype 5.

The reduction or elimination of the ability of the mutated adenovirus fiber protein to bind CAR as compared to the corresponding wild-type fiber protein is measured by comparing the transduction efficiency (gene transfer and expression of a marker gene) of an adenovirus particle containing the mutated fiber protein compared to an adenovirus particle containing the wild-type fiber protein for cells having CAR. As used herein, the term "substantially eliminate" refers to a transduction efficiency less than about 11% of the efficiency of the wild-type fiber containing virus on Hela cells using the transient transfection/infection system described in Example 1. Preferably, the efficiency is less than about 9%. Most preferably, the efficiency is less than about 8%. As used herein, the phrase "reduce" or "reduction" refers to a change in the efficiency of transduction by the adenovirus containing the mutated fiber as compared to the adenovirus containing the wild-type fiber to a level of about 75% or less of the wild-type on Hela cells using the transient transfection/infection system described in Example 1. Preferably, the change in efficiency is to a level of about 65% or less than wild-type. Most preferably, it is about 55% or less.

The fiber proteins of the invention are modified by chemical and biological techniques known to those skilled in the art. Such techniques permit the mutation of at least one amino acid in the CD loop of the wild-type fiber protein to change the ability of the protein to bind to CAR. As used herein, the term "mutate" or "mutation" or similar terms refers to the deletion or change of at least one amino acid in this part of the protein. The

amino acid can be changed by substitution or by modification in a way that derivatizes the amino acid.

As mentioned above, the preferred fiber protein of the invention is a mutated adenovirus serotype 5 fiber protein. The amino acid sequence of the wild-type protein is shown in SEQ ID NO:2. The CD loop in the wild-type adenovirus 5 protein extends from the amino acid at position 441 to the amino acid at position 453. Preferably, the amino acid at position 441 and/or the amino acid at position 442 of the wild-type fiber protein is mutated. Such mutation may involve a deletion of the amino acid at either or both of positions 441 and 442 (SEQ ID NOS:6, 10, 12, 13). Alternatively, substitution at either or both of these positions may be made. In a particularly preferred embodiment, the amino acid at position 441 of the wild-type fiber protein is changed from valine to alanine. In another particularly preferred embodiment, the amino acid at position 442 of the wild-type fiber protein is changed from lysine to alanine. Most preferably, the amino acid at position 441 of the wild-type fiber protein is changed from valine to alanine, and the amino acid at position 442 of the wild-type fiber protein is changed from lysine to alanine (SEQ ID NO:14).

The present inventors have also discovered that certain mutations in other parts of the wild-type adenovirus 5 fiber protein reduce or substantially eliminate the ability of an adenoviral particle with the mutated fiber to bind to CAR. In a preferred embodiment, the mutations are at one or more of amino acid positions 408, 409, 460, 509, 510, 538, and 539 of the wild-type protein. In one particularly preferred embodiment, the fiber protein is mutated at amino acid positions 408 and 409, preferably by substituting glutamic acid for serine at position 408 and substituting alanine for proline at position 409 (SEQ ID NO:4). In another preferred embodiment, the fiber protein is mutated at amino acid position 460 of the wild-type fiber protein, most preferably by substituting glutamic acid for arginine (SEQ ID NO:16). In another preferred embodiment, the fiber protein is mutated at at least one of amino acid positions 509 and 510 of the wild-type fiber protein, preferably by deleting the amino acids at both positions (SEQ ID NO:18). In another preferred embodiment, the fiber protein is mutated at at least one of amino acid positions 538 and 539 of the wild-type fiber protein, preferably by deleting the amino acids at both positions (SEQ ID NO:20).

Any or all of these mutations may be combined with mutations in the CD loop of adenovirus 5. In a preferred embodiment, the mutated fiber protein of the invention comprises at least one mutation at amino acid positions 441 and 442 of the wild-type fiber protein plus a mutation at one or more of amino acid positions 408, 409, 460, 509, 510, 538, and 539 of the wild-type fiber protein. For example, SEQ ID NO:8.

In an alternative, preferred embodiment, the mutated adenoviral fiber protein of the invention is a fiber protein where at least one amino acid in the AB loop of a wild-type fiber protein of an adenovirus from subgroup C, subgroup D, subgroup E, or selected viruses from subgroup F (in particular those having a long fiber), have been mutated to reduce or substantially eliminate the ability of the fiber protein to bind to CAR. In this embodiment, the preferred fiber protein of the invention is a mutated adenovirus serotype 5 fiber protein.

More preferably, the mutated adenovirus serotype 5 fiber protein contains mutations at amino acid positions 408 and/or 409 of the wild-type fiber protein. Preferably, the mutations are at both positions. As mentioned previously, such mutations may be deletions, substitutions, or a modification in a way that derivitizes the amino acid. The same type of mutation need not be made at each position. In one preferred version of this preferred embodiment, glutamic acid is substituted for serine at position 408. In an alternative preferred version of this preferred embodiment, alanine is substituted for proline at position 409. Most preferably, glutamic acid is substituted for serine at position 408, and alanine is substituted for proline at position 409 (SEQ ID NO:4).

The invention also comprises polynucleotides that encode the proteins of the invention. As used herein, the term "polynucleotide" means a nucleic acid molecule, such as DNA or RNA, that encodes a polynucleotide. The molecule may include regulatory sequences. Preferably, the polynucleotide is DNA. Such polynucleotides are prepared or obtained by techniques known by those skilled in the art in combination with the teachings contained therein. Examples of such polynucleotides are shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19. The polynucleotides of the invention also include polynucleotides that differ in certain bases but still encode the proteins of the invention due to the redundancy of the genetic code.

The invention further comprises vectors including the polynucleotides of the invention. Such vectors include partial or complete adenoviral genomes and plasmids. Such vectors are constructed by techniques known to those skilled in the art.

One of the principal uses of such vectors is the production of adenoviral packaging cells. The packaging cells of the invention are cells that provide complementing functions to the functions provided by the genes in the adenovirus genome that are to be packaged into the adenovirus particle. The production of such particles require that the genome be replicated and that those proteins necessary for assembling an infectious virus be produced. The particles may also require certain proteins necessary for the maturation of the viral particle. Such proteins may be provided by the vector or by the packaging cell.

The packaging cells of the invention may contain the polynucleotide encoding the mutated fiber protein. Such polynucleotide may be transfected into the cell, preferably as part of a plasmid, or it may be infected into the cell with a viral vector. It may be stably incorporated into the genome of the cell, thus providing for a stable cell line. Alternatively, it may be unincorporated into the genome, in which case a transient complementing cell will be provided.

The adenovirus genome to be packaged is transferred into the complementing cell by techniques known to those skilled in the art. These techniques include transfection or infection with another virus. The polynucleotide encoding the mutated fiber protein may be in this genome instead of in the packaging cell.

In certain cases, it may be desirable, when the polynucleotide encoding the mutated fiber is in the genome to be packaged, for the packaging cell to also encode a fiber protein. Such protein may assist in the maturation and packaging of an infectious particle. Such protein may be a wild-type fiber protein or one modified so as to be unable to attach to the penton base protein.

The packaging cells are cultured under conditions that permit the production of the desired viral particle. The viral particles are recovered by standard techniques.

A preferred way of making the adenoviral particles of the invention is as follows. The polynucleotide encoding the mutated fiber protein is made using standard techniques in an adenoviral shuttle plasmid. This plasmid contains the right end of the virus, in particular from the end of the E3 region through the right ITR. It also contains a recombinase site, such as a lox site. This plasmid is co-transfected into a complementing cell line along with a helper plasmid, which contains the remaining portion of the adenovirus genome, except for the E1 region and sometimes also the E2a region. A third plasmid, which is an expression plasmid containing a gene encoding a recombinase such as Cre, is also transfected into the complementing cell. The complementing cell is preferably a 293 cell, which contains the adenoviral E1 genes, or an AE1-2a cell {Gorziglia, Kadan, et al. 1996}, which contains the adenoviral E1 and E2a genes. Most preferably, the complementing cell is a 633 cell {Von Seggern, Huang, et al. 2000}, which stably expresses the adenovirus serotype 5 wild-type fiber protein, and was derived from the AE1-2a cell line.

The transfected complementing cells are maintained under standard cell culture conditions. The adenoviral plasmids recombine to form the adenoviral genome that is packaged. The particles are infectious, but replication deficient because their genome is missing at least the E1 genes. The particles contain both wild-type and mutated fiber

proteins. They are recovered from the crude viral lysate and are purified by standard techniques.

The recovered particles are preferably used to infect 293 or AE1-2a cells. This permits the recovery of particles whose capsids contain only the desired mutated fiber. This two-step procedure provides high titer batches of the adenoviral particles of the invention.

The adenoviral particles may be replication competent or replication incompetent. In a preferred embodiment of the invention, the particles selectively replicate in certain predetermined target tissue but are replication incompetent in other cells and tissues. In a particularly preferred embodiment of the invention, the adenoviral particles replicate in abnormally proliferating tissue, such as solid tumors and other neoplasms. Such replication conditional adenoviral particles and vectors may be produced by techniques known to those skilled in the art, such as those disclosed in the above-referenced U.S. Patent Nos. 5,998,205 and 5,801,029. These particles and vectors may be produced in adenoviral packaging cells as disclosed above. The preferred packaging cells are those that have been designed to limit homologous recombination that could lead to wild-type adenoviral particles. Such cells are disclosed in U.S. Patent Nos. 5,994,128, issued November 30, 1999 to Fallaux, et al., and 6,033,908, issued March 7, 2000 to Bout, et al. The packaging cell known as PER.C6, which is disclosed in these patents, is particularly preferred.

Preferably, the modified fiber polynucleotide also includes sequences that encode a targeting ligand. Accordingly, such sequences are transfected into the complementing cell by the shuttle plasmid.

Alternatively, the targeting ligand sequences may be included in the penton or hexon proteins. In such cases, they would be in the helper plasmid.

The packaged adenoviral genome may also contain a heterologous polynucleotide. This polynucleotide is usually included in the deleted E1 region of the helper plasmid. Alternatively, the polynucleotide may be in the E3 region, in which case it is included in the shuttle plasmid.

The adenovirus particles of the invention include the mutated fiber proteins. Such particles may include different types of the mutated fibers of the invention. They may also include wild-type fibers along with the mutated fibers.

The adenoviral particles of the invention preferably further comprise a targeting ligand included in a capsid protein of the particle. The ligand may be included in any of the capsid proteins, such as fiber, hexon, or penton. Preferably, the ligand is included in a fiber protein, which is preferably a mutated fiber protein of the invention. Most preferably, the

targeting ligand is included within the HI loop of the fiber protein. Any ligand that can fit in the HI loop and still provide a functional virus may be used. Such ligands may be as long as 80-90 amino acids. Such ligands are added by techniques known in the art, such as those disclosed in PCT application PCT/US99/02549, published as WO99/39734 on August 12, 1999, and Example 12 of U.S. patent application number 09/482,682, filed January 14, 2000, the disclosures of both of which are hereby incorporated herein by reference.

A targeting ligand may be any chemical moiety that preferentially directs the adenoviral particle to a desired cell type. The categories of such ligands include peptides, polypeptides, single chain antibodies, and multimeric proteins. Specific ligands include the TNF superfamily of ligands which include tumor necrosis factors (or TNF's) such as, for example, TNF-alpha and TNF-beta, lymphotoxins (LT), such as LT-α and LT-β, Fas ligand which binds to Fas antigen; CD40 ligand, which binds to the CD40 receptor of B-lymphocytes; CD30 ligand, which binds to the CD30 receptor of neoplastic cells of Hodgkin's lymphoma; CD27 ligand, NGF ligand, and OX-40 ligand; transferrin, which binds to the transferrin receptor located on tumor cells, activated T-cells, and neural tissue cells; ApoB, which binds to the LDL receptor of liver cells; alpha-2-macroglobulin, which binds to the LRP receptor of liver cells; alpha-1 acid glycoprotein, which binds to the asialoglycoprotein receptor of liver; mannose-containing peptides, which bind to the mannose receptor of macrophages; sialyl-Lewis-X antigen-containing peptides, which bind to the ELAM-1 receptor of activated endothelial cells; CD34 ligand, which binds to the CD34 ligand, which binds to the CD34 receptor of hematopoietic progenitor cells; ICAM-1, which binds to the LFA-1 (CD11b/CD18) receptor of lymphocytes, or to the Mac-1 (CD11a/CD18) receptor of macrophages; M-CSF, which binds to the c-fms receptor of spleen and bone marrow macrophages; circumsporozoite protein, which binds to hepatic *Plasmodium falciparum* receptor of liver cells; VLA-4, which binds to the VCAM-1 receptor of activated endothelial cells; HIV gp120 and Class II MHC antigen, which bind to the CD4 receptor of T-helper cells; the LDL receptor binding region of the apolipoprotein E (ApoE) molecule; colony stimulating factor, or CSF, which binds to the CSF receptor; insulin-like growth factors, such as IGF-I and IGF-II, which bind to the IGF-1 and IGF-II receptors, respectively; Interleukins 1 through 14, which bind to the Interleukin 1 through 14 receptors, respectively; the Fv antigen-binding domain of an immunoglobulin; and cyclic RGD peptide. Cyclic RGD (cRGD) is preferred. As used herein, the term "cyclic RGD" (or cRGD) refers to any amino

acid that binds to α integrins on the surface of cells and contains the sequence RGD (Arg-Gly-Asp). The sequence in SEQ ID NO:43 is particularly preferred.

In a preferred embodiment, because the adenovirus fiber has a trimeric structure, the ligand also has a trimeric structure. In a more preferred embodiment, the ligand is selected from the TNF superfamily of ligands hereinabove described. Such ligands are trimeric and of similar size to the fiber head domain. Such ligands may be incorporated into the fiber protein using the techniques disclosed in U.S. Patent No. 5,756,086, issued May 26, 1998 to McClelland et al., the disclosure of which is incorporated herein by reference.

The adenovirus particles may further include at least one heterologous polynucleotide. As used herein, the term "heterologous polynucleotide" means a polynucleotide derived from a biological source other than an adenovirus, which encodes a polypeptide when the adenovirus infects a cell. Such polynucleotides are included in the adenoviral genome within the particle and are added to that genome by techniques known in the art. Any heterologous polynucleotide of interest may be added. A preferred polynucleotide is one that encodes an immunostimulating protein, such as an interleukin, interferon, or colony stimulating factor. Mammalian GM-CSF is preferred. Preferably, such GM-CSF is a primate GM-CSF; most preferably, it is human GM-CSF. An alternative preferred polynucleotide encodes herpes simplex virus thymidine kinase (HSV-TK), which is useful as a safety switch as described in U.S. Patent Application No. 08/974,391, filed November 19, 1997, which published as PCT Publication No. WO/9925860, the disclosure of which is incorporated herein by reference.

The adenoviral particles of the invention are used to genetically engineer a cell to express a protein that it otherwise does not express or does not express in sufficient quantities. This is accomplished by infecting the desired cell with an adenoviral particle of the invention whose genome includes a desired heterologous polynucleotide. This permits the expression of the heterologous polynucleotide in the cell. Preferably, the cell is a mammalian cell. More preferably, the mammalian cell is a primate cell. Most preferably, the primate cell is a human cell. The cell may be inside the body of the animal (*in vivo*) or outside the body (*in vitro*).

Preferably, the adenoviral particle includes a targeting ligand as described above. This permits the delivery of a gene to a desired cell type which is different from the cell type that wild-type adenovirus particle infect or the same as a wild-type particle would infect but allowing the infection in a selective manner, i.e., non-target cell types would not be infected.

Thus, the adenoviruses of the invention can be used to study cell transduction and gene expression in vitro or in various animal models. The latter case includes *ex vivo* techniques, in which cells are transduced *in vitro* and then administered to the animal. They may also be used to conduct gene therapy on humans or other animals. Such gene therapy may be *ex vivo* or *in vivo*. For *in vivo* gene therapy, the adenoviral particles of the invention in a pharmaceutically-acceptable carrier are delivered to a human in a therapeutically effective amount in order to prevent, treat, or ameliorate a disease or other medical condition in the human through the introduction of a heterologous gene that encodes a therapeutic protein into cells in such human. The adenoviruses are delivered at a dose ranging from approximately 1 particle per kilogram of body weight to approximately 10^{14} particles per kilogram of body weight. Preferably, they are delivered at a dose of approximately 10^{6} particles per kilogram of body weight to approximately 10^{13} particles per kilogram of body weight. Most preferably, the dose ranges from approximately 10^{9} particles per kilogram of body weight to approximately 10^{12} particles per kilogram of body weight.

The adenoviral particles of the invention with the above-identified modifications in the AB loop, particularly those with modifications at amino acid positions 408 and 409 of the wild-type adenovirus serotype 5 fiber protein, and most particularly those where glutamic acid is substituted for serine at position 408 and alanine is substituted for proline at position 409 (SEQ ID NO:4), have additional desirable utilities. The inventors have unexpectedly discovered that such viral particles provide enhanced gene transfer to and expression in hepatocytes in the liver of an animal as compared to adenoviral particles with the wild-type fiber protein. Therefore, the invention includes a method of enhancing adenoviral-mediated gene transfer to and expression in cells in the liver of an animal by administering adenoviruses having such AB loop modification in at least one of their fiber proteins to an animal under conditions where cells in the liver are transduced. The hepatocytes are the cells that are primarily transduced. Preferably, an adenovirus particle comprising a mutated adenovirus serotype 5 fiber protein, wherein glutamic acid is substituted for serine at amino acid position 408 and alanine is substituted for proline at amino acid position 409 (SEQ ID NO:4), is used to deliver the heterologous gene.

Such adenoviral particles would be particularly useful for gene therapy where it is desired to express a heterologous gene in a patient's liver. This could be used, for example, in the treatment of diabetes, hemophilia, and diseases related to increased cholesterol or triglyceride blood levels in a patient such as atherosclerosis. It would also include anti-

angiogenesis treatment methods involving the delivery of one or more anti-angiogenic genes to the hepatocytes of a patient's liver.

Because of the enhanced gene transfer and expression, lower doses of these viral particles would be able to be used. The dose for these types of particles would be approximately 1 particle per kilogram of body weight to approximately 10^{13} particles per kilogram of body weight. Preferably, the dose would be approximately 10^5 particles per kilogram of body weight to approximately 10^{12} particles per kilogram of body weight. Most preferably, the dose ranges from approximately 10^8 particles per kilogram of body weight to approximately 10^{11} particles per kilogram of body weight.

Such particles are delivered by routes of administration known to those skilled in the art. One such route is intravenous injection. An alternative route is intraparenchymal injection. The particles may also be delivered by injection into the hepatic artery, portal vein, or bile duct.

Another use of the particles with the AB loop mutations would be as a basis for further modification of the particles, wherein the RGD amino acid sequence in the penton protein is deleted, modified, or substituted. Techniques for such modifications are disclosed in U.S. Patent Nos. 5,559,099, issued September 24, 1996 to Wickham, et al., 5,712,136, issued January 27, 1998 to Wickham, et al., and 5,731,190, issued March 24, 1998 to Wickham, et al., the disclosures of which are incorporated herein by reference. This would prevent the particles from binding to cell surface integrins and being taken into the cell.

EXAMPLES

Example 1
Adenovirus Type 5 Viral Particles Pseudotyped With Mutagenized Fiber Proteins Show Diminished Infectivity of Coxsackie B-Adenovirus Receptor-Bearing Cells

INTRODUCTION

The great interest in human adenovirus type 5 (Ad5) as a gene delivery platform is due in part to its ability to efficiently infect many cell types. Its wide tropism is mediated by a primary interaction between the Ad5 capsid protein, fiber, and its high-affinity cellular receptor, the coxsackie adenovirus receptor (CAR). Fiber is a homotrimeric protein present twelve times on the viral capsid. It has three domains: an N-terminal tail that interacts with the penton base in the viral capsid, a rod-like shaft containing 22 copies of a 15 amino-acid

beta sheet structure, and a globular knob domain. It is the knob domain that mediates binding to CAR during cell attachment. After the initial binding event, a second, low-affinity interaction takes place between the penton base and α_v integrins on the cell surface. This step is required for virus internalization and subsequent gene transfer.

There are many cases where it is desirable to deliver therapeutic genes to a subset of cell types. For this reason, there has been much effort to specifically target Ad5 vectors. This capability involves the detargeting away from its natural receptor and the simultaneous retargeting of the viral tropism toward a given cell type. The resulting vector would represent an important step in the development of this gene therapy platform, both from an efficacy and a safety standpoint. Reducing the undesired virus-tissue interactions and increasing the intended interaction would allow lower viral doses to be used and thereby potentially minimize the associated toxic side effects and host immune response.

Several strategies have been used to alter the receptor tropism and binding specificity of adenoviral vectors. These strategies include replacing the fiber knob domain with a knob from another Ad serotype with a different receptor specificity {Stevenson, Rollence, et al. 1995}{Krasnykh, Mikheeva, et al. 1996}{Stevenson, Rollence, et al. 1997}, the insertion of peptides onto the C-terminus of fiber {Wickham, Granados, et al. 1990}{Michael, Hong, et al. 1995}{Wickham, Tzeng, et al. 1997}or the exposed HI loop {Krasnykh, Dmitriev, et al. 1998} and the use of bifunctional antibodies {Wickham, Segal, et al. 1996}. The results of these efforts have been an expansion of viral tropism, which is suitable for some gene therapy applications such as vascular gene therapy where the aim is to improve the gene transfer efficiency of Ad vectors that are delivered locally. However, to specifically transduce certain cell types with systemically-delivered adenoviral vectors, it will be necessary to ablate the natural receptor tropism in combination with the introduction of a high affinity targeting ligand.

The analysis of multiple fiber mutations in the context of a viral particle remains a tedious process that involves the time-consuming incorporation of modified fiber genes into the adenoviral genomic DNA. Furthermore, the incorporation of mutated fiber genes into the Ad genome may affect the efficient growth and propagation of Ad. As a result, the generation and evaluation of adenoviral vectors containing mutated fiber proteins may require alternative means of growing the vectors that will allow for the efficient production of high titer viral stocks.

We have developed a novel system to rapidly analyze modified fiber proteins for desired tropism in the context of the viral particle. This system is based on the ability to pseudotype a fiberless Ad5 mutant with fiber proteins expressed transiently from an episomal plasmid (Fig. 1). The fiber-deleted Ad vector is Ad5.βgal.ΔF, which is an E1- E3- and fiber-gene deleted adenovirus that expresses cytoplasmic β-galactosidase under the control of the SV40 promoter {Von Seggern, Chiu, et al. 1999} (Fig. 1A). The modified fiber proteins for pseudotyping are produced from expression plasmid constructs designed for high levels of fiber protein expression {Von Seggern, Huang, et al. 2000}. The primary advantage of this system is that modified fiber proteins can be quickly incorporated into virions and functionally analyzed in their most relevant context for their effect on CAR interaction and subsequent gene transfer and expression. We used this system to analyze a panel of fiber mutants for their ability to mediate adenoviral gene transfer to Hela cells, a CAR-expressing cell line. We have shown that the transient transfection/infection system can efficiently pseudotype a fiberless viral capsid with levels of fiber protein indistinguishable from those seen on wildtype virions. We used this system to identify multiple fiber gene mutations that significantly reduce the ability of adenovirus to transduce cells and achieve gene transfer.

MATERIALS AND METHODS

Plasmids and fiber gene mutagenesis. The Ad5 fiber cDNA has been cloned into pcDNA3.1 to generate pDV60, as previously described {Von Seggern, Huang, et al. 2000}. Briefly, pDV60 contains the CMV promoter, the first Ad5 tripartite leader exon (TPL), the natural first intron and the fused second and third TPL exons upstream of the Ad5 fiber gene. All amino acid changes were incorporated into the fiber cDNA using the pDV60 plasmid as the template. Individual amino acid residues in pDV60 were mutagenized using the QuickChange Site-Directed Mutagenesis system (Stratagene, La Jolla CA). The oligonucleotide primers used for the incorporation of amino acid changes are listed in Table 1 for each single or double amino acid modification. The thermal cycler protocol was 95°C for 30 sec, followed by 18 cycles of 95°C for 30 sec, 55°C for 1 min, and 68°C for 20 min.

The entire knob domain of the Ad5 fiber was deleted from amino acids 404 to 581. For restoration of trimerization of the fiber tail and shaft, a 31 amino acid peptide derived from the GCN4 leucine zipper {Harbury, Zhang, et al. 1993} was fused immediately after the fiber TLWT sequence at the fiber shaft-head junction using PCR gene overlap extension {Horton, Cai, et al. 1990}. This reaction fused the Ad5 fiber tail and shaft regions (amino

acids 1 to 403) to the GCN4 isoleucine 31 amino acid peptide to form the KO11 mutant and was cloned into pDV60 to create pDKO11. For all fiber mutations, the nucleotide sequence of the cloned insert was determined and in each case a clone having the expected sequence was selected. The pDV55 control plasmid is similar to pDV60, except that it lacks the fiber gene {Von Seggern, Huang, et al. 2000}.

Viruses. Ad5.βgal.wt is a first generation E1-, E3-deleted adenovirus containing a lacZ reporter cassette in the E1 region {Von Seggern, Chiu, et al. 1999}. Ad5.βgal.ΔF is identical to Ad5.βgal.wt except that the fiber gene is deleted {Von Seggern, Chiu, et al. 1999}.

Cells. Human 293T cells were obtained from ATCC (CRL 11268) and were cultured in the DMEM containing 10% FBS. The 293T cells stably express the SV40 large T antigen that allows for the amplification of plasmids from the SV40 origin of replication. The 633 cells stably express the Ad5 fiber protein {Von Seggern, Huang, et al. 2000} and are derived from AE1-2a, a cell line that complements E1a- and E2a-deleted adenoviral vectors {Gorziglia, Kadan, et al. 1996}. 633 cells were grown in Richter's CM (Life Technologies #C-2671) and 10% FBS. Hela cells (ATCC CCL-2) were grown in Dulbecco's modified Eagle's media supplemented with 10% FBS. For a further description of the preparation of AE1-2a cells, also known as S8 cells, and the preparation of 633 cells, see Example 6F and 6G of U.S. Patent Application number 09/482,682, filed January 14, 2000, which disclosure is incorporated herein by reference.

Transient Transfection/Infection. Mutated fiber proteins were incorporated into adenoviral particles using the transient transfection/infection system. For each virus preparation using the transient transfection/infection system, four 15 cm dishes of 70% confluent 293T cells were used. For transfections, 100ug of each fiber expression plasmid DNA listed in Table 2, 400ul lipofectamine (Life Technologies, Rockville, MD) and 3.6ml Opti-MEM 1 media (Life Technologies, Rockville, MD) were combined in a conical 250ml sterile bottle. At the end of a 30 minute room temperature incubation, 60ml Opti-MEM 1 media was added. A 16ml aliquot of this transfection mix was added to each plate and incubated at 37°C, 5% CO_2 for 5 hours. The transfection media was then aspirated and 20 ml of complete DMEM media was added. The dishes were then incubated at 37°C, 5% CO_2 for 24 hours to allow expression of the fiber protein.

The transfected 293T cells were then infected with Ad5.βGal.ΔF/F$^+$ virus at a particle per cell ratio of 350. The Ad5.βGal.ΔF/F$^+$ virus is an E1, E3, fiber-deleted Ad5 vector {Von

Seggern, Chiu, et al. 1999} that was propagated in the fiber-complementing cell line, 633, such that the capsid contains wildtype Ad5 fiber protein {Von Seggern, Huang, et al. 2000}. The growth media was aspirated and 2.5ml of infection media (DMEM and 2% FBS) containing Ad5.βGal.ΔF/F^+ was added and slowly rocked at 37^0C, 5% CO_2 for 2 hours. Twenty ml of growth media was then added (DMEM and 10% FBS) and the plates were incubated at 37^0C, 5% CO_2 overnight. The media was replaced the next day and the incubation was continued until complete cytopathic effect (CPE) was observed, typically in about 3 to 4 days. The transfected/infected 293T cells were harvested after complete CPE by gently dislodging the cells, pelleting by centrifugation, and resuspending in 1ml phosphate buffered saline. A crude viral lysate (CVL) was prepared by five freeze-thaw cycles to disrupt the cells and release the virus. The virus was purified by CsCl gradient centrifugation using standard procedures. The virus particle titer was determined spectrophotometrically as described {Mittereder, March, et al. 1996}. Yields of Ad5.βGal.ΔF virus pseudotyped with modified fiber protein typically ranged from 10^{11} to 10^{12} particles.

Western immunoblot analysis. The expression and incorporation of each fiber protein onto adenoviral particles was verified by denaturing sodium dodecyl-sulfate (SDS) 4 to 12% polyacrylamide gel electrophoresis (PAGE) and Western immunoblot analysis. An aliquot of each adenoviral vector preparation corresponding to 5.0×10^9 particles per lane was analyzed. The proteins were transferred to a nitrocellulose membrane with a minitransblot apparatus (Novex Inc.) for 90 minutes at 30V. The membrane was blocked for at least 1 hour at room temperature in 10mM Tris, pH7.4 containing 150mM NaCl, 2mM EDTA, 0.04% Tween-20, and 5% dried milk. The blocked membrane was incubated for 1 hour with a 1:1000 dilution of a primary rabbit anti-Ad5 fiber polyclonal antiserum. The membrane was then developed with a 1:5000 dilution of the secondary donkey anti-rabbit IgG horseradish peroxidase-conjugated antibody (Amersham Lifesciences, Arlington Heights, IL) using an enhanced chemiluminescense system (Amersham Lifesciences). The membrane was exposed to film for approximately 1 to 20 seconds. The membrane was then used to reprobe for detection of the adenoviral penton protein to ensure equivalent loading of viral particles. Briefly, the membrane was incubated for 1 hour with a 1:5000 dilution of the primary rabbit anti-Ad5 penton polyclonal antiserum. The membrane was then re-developed with a 1:5000 dilution of the secondary goat anti-rabbit IgG horseradish peroxidase-conjugated antibody as described above.

Production of anti-Ad5 fiber and anti-Ad5 penton-specific antiserum. Both of the rabbit primary antibodies used in the anti-fiber and anti-penton Western immunoblot analysis were generated by immunizations of New Zealand White rabbits (Loftstrand Labs, Ltd., Gaithersburg, MD). The Ad5 fiber and penton proteins were expressed using the baculoviral expression system. The purified Ad5 fiber protein and partially purified penton base proteins were used for immunizations according to standard protocols. The antiserum obtained was tested for immunoreactivity against the Ad5 fiber and penton proteins by Western immunoblot analysis.

Adenoviral transduction. Hela cells were infected with the adenoviral vectors containing mutated fiber proteins to evaluate the effects of fiber amino acid mutations on CAR interaction and subsequent gene expression. Monolayers of HeLa cells in 12 well dishes were infected with 1000 particles per cell for 2 hours at 37^0C in a total volume of 0.35 ml of the DMEM containing 2% FBS. The infection medium was then aspirated from the monolayers and 1ml of complete DMEM containing 10% FBS was added per well. The cells were incubated for an additional 24 hours to allow for β-galactosidase expression.

β-galactosidase expression analysis. The expression of β-galactosidase encoded by the adenoviral vectors in the infected cells was measured by a chemiluminescence reporter assay and by histochemical staining with a chromogenic substrate. The relative levels of β-galactosidase activity were determined using the Galacto-Light chemiluminescence reporter assay system (Tropix, Bedford, MA). Briefly, the cell monolayers were washed with PBS and processed according to the manufacturer's protocol. The cell homogenate was transferred to a microfuge tube and centrifuged to remove cellular debris. Total protein concentration was determined using the bicinchoninic acid (BCA) protein assay (Pierce, Inc., Rockford, IL) with bovine serum albumin as the assay standard. An aliquot of each sample was then incubated with the Tropix β-galactosidase substrate for 45 minutes in a 96 well plate. A luminometer was used to determine the relative light units (RLU) emitted per sample and then normalized for the amount of total protein in each sample (RLU/ug total protein). For the histochemical staining procedure, the cell monolayers were fixed with 0.5% glutaraldehyde in PBS, and then were incubated with a mixture of 1 mg of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) per ml, 5mM potassium ferrocyanide, 5mM potassium ferricyanide and 2 mM $MgCl_2$ in 0.5 ml of PBS. The monolayers were washed with PBS and the blue cells were visualized by light microscopy with a Zeiss ID03 microscope.

RESULTS

Transient transfection/infection system. To rapidly evaluate a panel of potential CAR-binding fiber mutants in the context of viral particles, we have developed a transient transfection/infection system. This system, which is based on pseudotyping a fiberless virus with the mutant fiber proteins, consists of two components. The first is an E1, E3, fiber-deleted adenovirus, Ad5.βgal.ΔF {Von Seggern, Chiu, et al. 1999} (Fig. 1A). This virus, when grown on a non fiber-complementing cell line such as 293T, yields viral particles lacking fiber protein. For purposes here, these fiberless virions are designated Ad5.βgal.ΔF/F^{-}. If Ad5.βgal.ΔF is produced on the fiber-complementing cell line 633 {Von Seggern, Huang, et al. 2000}, the virions contain a full complement of wildtype fiber protein on the surface and is referred to as Ad5.βgal.ΔF/F^{+}. The second component of the system is an expression plasmid that supplies fiber protein to the assembling virus in *trans*. This plasmid, pDV60, is designed to express high levels of fiber protein {Von Seggern, Huang, et al. 2000}.

The transient transfection/infection system is shown schematically in Fig. 1B. Transfection of 293T cells by the pDV60-based fiber-expression plasmid results in high levels of fiber production in the cells. Twenty-four hours later, the cells are infected with Ad5.βgal.ΔF/F^{+} that has been previously pseudotyped with wildtype fiber by growth in 633 cells. Approximately three days later, the infected cells are collected and viral particles, now pseudotyped with the fiber protein supplied in *trans* by the fiber-expression plasmid, are purified. In this way, any plasmid-encoded fiber proteins that are capable of trimerization and incorporating into the viral particles will complement Ad5.βgal.ΔF. Ad5.βgal.ΔF that is pseudotyped either by growth in 633 cells or by transient transfection with a fiber expression plasmid is designated Ad5.βgal.ΔF/F^{+}. The function of these modified fiber proteins in the context of a viral particle can then be tested for their ability to mediate fiber-dependent Ad infection and gene transfer.

To compare the level of fiber protein incorporated onto Ad5.βgal.ΔF/F^{+} viral particles generated by this system with the levels in Ad5.βgal.wt, Western immunoblot analysis was performed. Equal particle numbers of Ad5.βgal.ΔF/F^{-}, Ad5.βgal.ΔF/F^{+} pseudotyped by pDV60-encoded fiber protein, and Ad5.βgal.wt were evaluated for fiber and penton protein levels. As reported previously {Von Seggern, Chiu, et al. 1999}, the Ad5.βgal.ΔF/F^{-} virions lacked any detectable fiber protein, and Ad5.βgal.wt contained expected levels of the 62kDa

fiber protein. Importantly, the level of pDV60-encoded fiber protein incorporated into the Ad5.βgal.ΔF/F$^+$ pseudotyped virions using the transient transfection/infection system was equivalent to the level of fiber protein in the Ad5.βgal.wt particles. The equivalent loading of viral particles was demonstrated by detection of the 68kDa penton monomer for each vector. These results indicate that expression of fiber protein in *trans* from this expression plasmid can complement Ad5.βgal.ΔF and can result in a level of fiber protein on the capsid that is indistinguishable from that of an adenovirus containing fiber within its genome.

Fiber mutation analysis. The transient transfection/infection system was then used to evaluate a series of mutations in the fiber knob for their effect on CAR-mediated gene transfer of Ad5 particles. A panel of expression plasmids encoding fourteen mutant fiber proteins was constructed (Table 2). As controls, the wildtype fiber (pDV60) and a null construct (pDV55) were used {Von Seggern, Huang, et al. 2000}. These plasmids were transfected into 293T cells, followed by infection with Ad5.βgal.ΔF/F$^+$. The resulting virions obtained from this procedure were thus pseudotyped with the plasmid-encoded fibers. The expression and assembly of each fiber protein into the adenoviral capsid was examined by Western immunoblot analysis of the CsCl-purified virus stocks. The relative levels of fiber protein on the capsid were compared with the amount of penton protein to control for equal loading of viral particles in each lane. The fiber proteins encoded by most mutants were sufficiently expressed, trimerized and incorporated into the Ad5.βgal.ΔF viral particles and the 62kDa fiber monomer was detected in this analysis. Analysis of the KO11 mutant displayed the expected protein of approximately 48kDa although this truncated fiber protein was not incorporated to the same level as wild-type fiber. These results demonstrate that the mutations introduced into the majority of these fiber genes did not impair their ability to be expressed, trimerized and incorporated into viral particles at levels indistinguishable from wild-type fiber. However, mutants KO2 (SEQ ID NO:6), KO1+2 (SEQ ID NO:8), KO2a (SEQ ID NO:10), and KO11 showed lower levels of incorporated fiber protein although KO11 may have a reduced immunoreactivity with an antiserum that was generated against the full-length wildtype Ad5 fiber protein. Analysis of the relative expression level and trimerization ability of these mutants on non-denaturing polyacrylamide gels showed lower levels of fiber monomer and trimer, indicative of deficiencies in the steady-state levels of these mutant proteins. Except for KO11, which is a deletion of the entire knob, all of these mutants have a mutation at V441 in common.

Having demonstrated efficient expression in *trans* and virion incorporation of most of these mutant fiber proteins, we next evaluated the affects of these mutations on functional CAR-binding properties. We did this by comparing the transduction efficiency on Hela cells of virions pseudotyped with mutant fiber protein and those pseudotyped with wildtype fiber protein. Transduction efficiency was measured in two ways. A chemiluminescence reporter assay was used to measure the level of adenoviral-encoded□ β-galactosidase activity and the values (RLU/ug total cellular protein) from one representative experiment are shown in Table 2. A total of five to six separate transductions were performed and the mean □β-galactosidase activity values (RLU/ug total cellular protein) were calculated for each adenoviral vector containing the individual fiber mutants. These values were then normalized to the β-galactosidase activity chemiluminescence values obtained with the wildtype fiber to obtain the relative activity of each mutant compared to wildtype fiber. The F-, fiberless vector displayed the most significant, 1000-fold reduction in transduction with only 0.1% of wt activity demonstrating the need for fiber in the efficient transduction of HeLa cells. The KO1 mutation displayed approximately a 70-fold reduction resulting in only 1.4% wt β-galactosidase activity levels. The KO2 (SEQ ID NO:6) mutation resulted in an approximately 167-fold reduction with 0.6% wt activity. The KO2a (SEQ ID NO:10), KO2b (SEQ ID NO:12), and KO2c (SEQ ID NO:14) constructs were designed to identify the amino acid mutation responsible for the significant reduction in CAR interaction. In this comparison, it was revealed that the deletion of amino acid V441 reduced CAR interaction greatest as this single deletion in KO2a (SEQ ID NO:10) resulted in the 167-fold reduction of wt activity and the deletion of K442 had no further effect. The most potent mutation was found with combining the KO1 (SEQ ID NO:4) and KO2 (SEQ ID NO:6) mutations in the KO1+2 (SEQ ID NO:8) construct as this combination resulted in a 1000-fold reduction with only 0.1% wt levels found. A dramatic reduction in transduction efficiency was also found for the KO11 mutant with a 125-fold reduction of β-galactosidase activity when the entire knob domain was deleted. Significant decreases were also observed for KO3 (SEQ ID NO:16), KO4 (SEQ ID NO:18), and KO5 (SEQ ID NO:20) although not as dramatic. Little to no effect on fiber-mediated transduction and gene expression was noted for KO4+5, KO8, KO9 and KO10. The average percent wild-type β-galactosidase activity for each pseudotyped adenoviral vector is shown graphically in Figure 2.

We also analyzed the transduction efficiency of the pseudotyped Ad5.βgal.ΔF/F$^+$ by measuring the percentage of cells that were positive for the lacZ reporter gene. This was done

by staining the transduced cell monolayers with X-gal. For all mutants, the histochemical data was consistent with the chemiluminescence data. At 1000 particles per cell, Hela cells infected with Ad5.βgal.ΔF/F^{+} pseudotyped with pDV60 showed a high percentage of blue, X-gal positive cells, while Ad5.βgal.ΔF/F- pseudotyped with pDV55 demonstrated very few if any blue cells. The mutants KO1 (SEQ ID NO:4), KO2a (SEQ ID NO:10), and KO2 (SEQ ID NO:6) and KO2c (SEQ ID NO:14), which showed dramatically lower β-galactosidase activity (Table 2 and Fig. 2), also showed extremely low numbers of blue cells as expected. KO4 showed an intermediate reduction in β-galactosidase activity and in the number of X-gal stained positive cells while KO10 had little effect on transduction efficiency by either measure (Fig. 2).

DISCUSSION

One approach to selective cell transduction is to manipulate the fiber protein to redirect the receptor specificity to a particular cell type. There are two requirements for such a targeted adenovirus gene therapy vector. One is ablation of the natural tropism and the second is the introduction of a novel tropism to desired target cell types. We developed a novel system to analyze mutant fiber proteins in the context of the viral particle. Using this system, we identified a number of mutant fiber proteins that retained the ability to incorporate into viral particles but demonstrated a reduction in fiber-mediated gene transfer due to a diminished interaction with CAR. The most dramatic mutations were KO11 (Δ404-581) and the mutations localized to the fiber AB loop (KO1: S408E, P409A) (SEQ ID NO:4) and the CD loop (KO2: ΔV441,K442) (SEQ ID NO:6). We generated pseudotyped viral particles containing fiber proteins that incorporates both of these mutations and then directly demonstrated an effect on viral transduction efficiency. In addition, we have identified a novel region of the Ad5 fiber, in the CD loop, that is involved in mediating viral transduction. All mutants that incorporated amino acid changes within this region displayed a reduction in fiber-mediated gene transfer including KO1+2 (SEQ ID NO:8), KO2 (SEQ ID NO:6), KO2a (SEQ ID NO:10), KO1 (SEQ ID NO:4), KO2b (SEQ ID NO:12) and KO2c (SEQ ID NO:14) that resulted in a 1000 to 12-fold reduction. The KO1+2 mutation containing a two amino acid substitution in the A:B loop and a two amino acid deletion in the C:D loop demonstrated the most potent reduction in gene transfer which is greater than either mutation alone. These data suggest that there may be simply a disturbance in overall structure or a cooperative interaction in CAR binding between these two loop regions.

The KO2 (ΔV441,K442) (SEQ ID NO:6) and KO2a (ΔV441) (SEQ ID NO:10) mutants showed a significant decrease in transduction efficiency, greater than 160-fold. A portion of this reduction is undoubtedly due to the lower levels of the mutant fiber protein on the viral capsid. However, a significant reduction in transduction efficiency has also been observed for a virus that has the identical KO2 mutation introduced genetically into the viral genome. This virus has a full complement of the mutant fiber protein on the capsid and still shows a dramatic reduction in transduction efficiency in all cell types tested.

We found here that fiber proteins containing amino acid mutations Δ509-510 (KO4) (SEQ ID NO:18) and Δ538-539 (KO5) (SEQ ID NO:20) had reduced capability for transducing Hela cells compared to virus particles pseudotyped with wildtype fiber protein. This suggests that in the Ad5 fiber, these residues on the adjacent monomer are involved in CAR-binding.

The second requirement for an adenovirus that transduces in a cell-type specific manner is the introduction of a novel tropism. The most efficient means is by genetic modification of the fiber gene. Krasnykh et al. {Krasnykh, Dmitriev, et al. 1998} have shown that the HI loop is an appropriate location in the fiber protein to insert peptides with novel receptor specificities. For example, the cRGD ligand (SEQ ID NO:43) {Pasqualini, Koivunen, et al. 1995} inserted into the HI loop has been shown to expand the tropism of Ad both *in vitro* {Dmitriev, Krasnykh, et al. 1998} and *in vivo* {Reynolds, Dmitriev, et al. 1999}. One advantage of the transient transfection/infection system described here is that there is no need for a pseudoreceptor system to propagate virions that do not bind CAR. CAR binding is needed for efficient viral production. The production of high titer vector stocks containing ablated fiber-CAR interactions is difficult without an alternative cell-binding pathway. Virus production in the transient transfection/infection system involves a single round of replication that results in a viral capsid pseudotyped with fiber mutants expressed *in trans*. It should be possible therefore to more easily test combinations of CAR-binding mutations and targeting ligands for their ability to mediate transduction.

TABLE 1. Oligonucleotides used in Ad5 fiber gene mutagenesis.

Fiber expression plasmid		Oligonucleotide sequence	Fiber mutation[1]
pDKO1	Seq. ID 21	5'-ACCACACCAGCTCCAGAGGCTAACTGTAGACTAAATGC-3'	S408E, P409A
	Seq. ID 22	5'-GCATTTAGTCTACAGTTAGCCTCTGGAGCTGGTGTGTT-3'	
pDKO2	Seq. ID 23	5'-ACAGTTTCAGTTTTGGCCGGCAGTTTGGCTCCAATATC-3' 5'-	ΔV441, K442
	Seq. ID 24	GATATTGGAGCCAAACTGCCGGCCAAAACTGAAACTGT-3'	
pDKO2a	Seq. ID 25	5'-ACAGTTTCAGTTTTGGCTAAAGGCAGTTTGGCTCCA-3'	ΔV441
	Seq. ID 26	5'-TGGAGCCAAACTGCCTTTAGCCAAAACTGAAACTGT-3'	
pDKO2b	Seq. ID 27	5'-GTTTCAGTTTTGGCTGTTGGCAGTTTGGCTCCAATA-3'	ΔK442
	Seq. ID 28	5'-TATTGGAGCCAAACTGCCAACAGCCAAAACTGAAAC-3'	
pDKO2c	Seq. ID 29	5'-GTTTCAGTTTTGGCTGCTGCAGGCAGTTTGGCTCCA-3'	V441A, K442A
	Seq. ID 30	5'-TGGAGCCAAACTGCCTGCAGCAGCCAAAACTGAAAC-3'	
pDKO3	Seq. ID 31	5'-GCTCATCTTATTATAGAATTCGACGAAAATGGAGTG-3'	R460E
	Seq. ID 32	5'-CACTCCATTTTCGTCGAATTCTATAATAAGATGAGC-3'	
pDKO4	Seq. ID 33	5'-GCTTATCCAAAATCTCACACTGCCAAAAGTAACATTGTC-3'	ΔG509, K510
	Seq. ID 34	5'-GACAATGTTACTTTTGGCAGTGTGAGATTTTGGATAAGC-3'	
PDKO5	Seq. ID 35	5'-CTAACCATTACACTAAACCAGGAAACAGGAGACAC-3'	ΔG538, T539
	Seq. ID 36	5'-GTGTCTCCTGTTTCCTGGTTTAGTGTAATGGTTAG-3'	
PDKO8	Seq. ID 37	5'-ATAAGATTTGACGAAACTGGAGTGCTACTAAAC-3'	N464T
	Seq. ID 38	5'-GTTTAGTAGCACTCCAGTTTCGTCAAATCTTAT-3'	
PDKO9	Seq. ID 39	5'-TTTGACGAAAATGGACACCTACTAAACAATTCC-3'	V466H
	Seq. ID 40	5'-GGAATTGTTTAGTAGGTGTCCAGTTTCGTCAAA-3'	
PDKO10	Seq. ID 41	5'-AACCTATCAGCTTATGCAAAATCTCACGGTAAA-3'	P505A
	Seq. ID 42	5'-TTTACCGTGAGATTTTGCATAAGCTGATAGGT-3'	

[1]numbering of amino acid residues as in Xia et al 1994.

TABLE 2. Transduction efficiency of pseudotyped Ad5.βgal.ΔF/F^{+} on Hela cells.

Fiber expression plasmid	Fiber mutant		Representative avg □gal activity (RLU/□g protein)	Mean % wt (± SD)**
	Designation	Mutation[†]		
pDV60	Wildtype (wt)	None	529882.0	100.0 (±0.9)
pDV55	F	Null	266.3	0.1 (±0.1)*
pDKO1	KO1	S408E, P409A	7618.0	1.4 (±0.8)*
pDKO1+2	KO1+2	S408E, P409A, ΔV441, K442	472.3	0.1 (±0.0)*
pDKO2	KO2	ΔV441, K442	3323.7	0.6 (±0.2)*
pDKO2a	KO2a	ΔV441	4002.0	0.6 (±0.2)*
pDKO2b	KO2b	ΔK442	44399.0	8.3 (±0.6)*
pDKO2c	KO2c	V441A, K442A	53336.7	8.5 (±2.2)*
pDKO3	KO3	R460E	359229.7	63.3 (±9.1)*
pDKO4	KO4	ΔG509, K510	212987.7	38.2 (±2.8)*
pDKO5	KO5	ΔG538, T539	331349.7	58.3 (±7.1)*
pDKO4+5	KO4+5	ΔG509, K510, ΔG538, T539	499740.3	91.1 (±12.1)
pDKO8	KO8	N464T	469705.7	92.6 (±16.9)
pDKO9	KO9	V466H	391442.7	80.9 (±15.1)
pDKO10	KO10	P505A	447260.3	79.6 (±6.2)
pDKO11	KO11	Δ404-581	4521.0	0.8 (±0.1)*

RLU, relative light units. Value represents the average of three wells.

[†]numbering of fiber amino acid residues as in Xia et al. 1994.

**% wildtype represents the mean (±SD) of the βgal activity of Ad5.βgal.ΔF pseudotyped with each corresponding fiber mutant in 5-6 separate transductions. All values were normalized to wildtype (pDV60) at 100%.

**Significantly different from wt fiber using an unpaired, two-tailed t-test analysis, p<0.001*

Example 2
Description of an Av1nBg Virus Containing a Fiber With the cRGD Targeting Moiety and the ΔV441-K442 (KO2) CAR-binding Mutation

Plasmid Description: The following three plasmids were used to rescue infectious adenoviruses containing modified fibers. p5FloxPRGD is a shuttle plasmid used to incorporate modifed fibers into the Ad genome (Figure 3). It consists of the final 6kb of DNA from the right end of an Av1 genome. A lox site was inserted upstream of the fiber gene. In addition, the Ad5 packaging signal has been inserted near the right inverted terminal repeat (RITR). pAv1hlpr is a helper plasmid that consists of an entire Av1 genome with the exception of the right ITR (Figure 4). It has a reporter gene inserted in the E1 region that encodes a nuclear-localized ß-gal protein, the HSV thymidine kinase gene inserted in the E3 region, and a lox site inserted in a location identical to that in p5FloxPRGD. pCre is a plasmid that constitutively expresses the Cre recombinase. It consists of the Cre gene cloned into the expression plasmid pcDNA1.lzeo$^+$ (Invitrogen). None of these plasmids are capable of producing infectious Ad on their own. But when these three plasmids are cotransfected into a complementing cell line, the Cre protein mediates recombination between the lox sites in p5FloxPRGD and pAv1hlpr, reconstituting a full length Av1 genome, which is then capable of producing infectious virus.

Generation of KO2 fiber mutations: All amino acid changes were incorporated into the fiber gene using the p5FloxPRGD adenoviral shuttle plasmid as the template. This shuttle plasmid encodes a fiber that contains a cRGD peptide sequence, HCDCRGDCFC (SEQ ID NO:43), inserted in the HI loop. The cRGD peptide has been shown to bind to α_v-integrins on the cell surface. Amino acid residues V441 and K442 in the CD loop of the fiber gene were deleted using the QuickChange Site-Directed Mutagenesis system (Stratagene, La Jolla CA). Deletion of these residues has been shown in the transient transfection/infection system to dramatically inhibit transduction of the Hela cell line which expresses the adenoviral receptor, CAR The resulting shuttle plasmid was called pSKO2 (Figure 5).

Generation of adenoviral vectors with CD loop mutations: The mutagenized fiber gene was incorporated into the adenoviral DNA backbone by cre-lox recombination. To do this, the pSKO2 shuttle plasmid and the pAv1hlpr helper plasmid were cotransfected with pCre into 633 cells, a cell line that expressed wildtype fiber {Von Seggern, Huang, et al. 2000}. Expression of the Cre recombinase from pCre mediates recombination between lox

sites in pSKO2 and pAv1hlpr, resulting in full length Av1 viral DNA, with nuclear β-Gal transgene in the E1 region. In the 633 complementing cell line, this viral DNA is capable of being packaged into infectious viral particles containing a mixture of wildtype fiber and mutant fibers. Upon observation of cytopathic effect, the virus was purified by standard CsCl centrifugation procedures. This virus was designated Av1nBgHIRGDKO2(633). In order to obtain viral particles containing only the adenoviral-encoded mutant fiber gene with the ΔV441, K442 mutations (SEQ ID NO:6), this viral preparation was used to infect AE1-2a cells, which do not express fiber {Gorziglia, Kadan, et al. 1996}. Viral particles were purified as above. This virus was designated Av1nBgHIRGDKO2.

Comparable fiber incorporation in Av1nBgHIRGDKO2: To ensure that the levels of the mutant fiber on the Av1nBgHIRGDKO2 viral particles were normal relative to viruses with wildtype fiber levels, Western blot analysis was performed. Equivalent amounts of Av1nBg and Av1nBgHIRGDKO2 were subjected to SDS-PAGE. This gel was transferred to a membrane and incubated with rabbit anti-Ad5 fiber and rabbit anti-Ad5 penton polyclonal antisera. The fiber penton ratio on Av1nBgHIRGDKO2 viral particles is indistinguishable from that of Av1nBg, demonstrating that there was no effect of the fiber mutations on the level of fiber protein assembled on the viral capsid.

Transduction efficiency of Av1nBgHIRGDKO2: As shown previously in Example 1, adenoviruses pseudotyped with fiber proteins containing deletions of V441 and K442 in the CD loop are severely affected in their ability mediate gene transfer in the CAR-expressing cell line Hela. In order to test the idea that gene transfer by these mutant viruses can be mediated by alternative ligand/receptor interactions, we tested the ability of the Av1nBgHIRGDKO2 virus to transduce HDF, Hela, PC3 and CHO-K1 cell lines (Fig 6). All of these cells lines express α_v integrins on the cell surface and, with the exception of Hela, show poor transduction by adenovirus due to a known or presumed deficiency in CAR levels.

Transduction by Av1nBg, which has wildtype fiber, is extremely inefficient in HDF, PC3 and CHO-KI, as expected (SEQ ID NO:6). Av1nBgHIRGD, which contains RGD in the HI loop, transduces HDF, PC3 and CHO-K1 with much higher efficiency. However, when cRGD is placed in the context of the V441, K442 deletion, as in the Av1nBgHIRGDKO2 virus, transduction efficiency remains inefficient. The levels of Av1nBgHIRGDKO2 transduction in HDF and CHO-K1 cells were similar to Av1nBg and lower in PC3 cells.

This reduction in the inability of Av1nBgHIRGDKO2 to transduce cells through α_v-integrins is not due to a defect in the RGD targeting moiety. When Av1nBgHIRGDKO2 is

purified from 633 cells, the resulting virus, Av1nBgHIRGDKO2 (633) contains a mixture of the wildtype fiber expressed from the 633 cells, and the mutant fiber expressed from the adenoviral genome. This virus, which has both types of fiber on the virion surface, is now able to mediate efficient transduction of HDF cells. This indicates that the RGD in the fiber is able to mediate transduction of HDF cells, even in the context of the V441, K442 deletion.

To further demonstrate that the RGD in the fiber containing the V441, K442 deletion is functional, we performed competition experiments (Fig. 7). HDF cells were transduced with Av1GFPHIRGD in the presence of increasing amounts of a competitor virus Av1nBg, Av1nBgHIRGD or Av1nBgHIRGDKO2. The percent of GFP positive cells was then measured. All three competitor viruses successfully inhibited the ability of the Av1GFPHIRGD virus to mediate gene-transfer to comparable degrees.

We conclude from these experiments that the RGD retargeting ligand sequence in a fiber containing the V441, K442 deletion is functional in its ability to mediate binding of α_v-integrins (by its ability to inhibit transduction of Av1GFPHIRGD, see Fig. 4) and transduction of cells low in CAR (by its ability to transduce HDF cells when the virus is purified from 633 cells).

In summary, we have described a CD loop mutation, ΔV441 K442 (SEQ ID NO:6), that dramatically reduces the ability of viruses pseudotyped with this fiber protein to mediate gene transfer. Here we show that a viral genome containing the V441 K442 deletion and an RGD insertion in the fiber gene can be rescued. This virus was designated Av1nBgHIRGDKO2. The mutant fiber protein is incorporated into the viral particles at levels indistinguishable from wildtype. The RGD targeting ligand in Av1nBgHIRGDKO2 is functional, as shown in two experiments. First, Av1nBgHIRGDKO2 was able to compete Av1GFPHIRGD transduction of HDF cells which are low in CAR and high in α_v integrins. Second, we showed that retargeting of Av1nBgHIRGDKO2 through the RGD targeting ligand in HDF cells can be achieved but was dependent on the propagation of the virus in cells expressing wildtype fiber. We conclude that the V441 K442 deletion has dramatically reduced ability to mediate transduction of CAR-expressing cells and that fibers containing these deletions and alternatives targeting ligands are functional.

Example 3
Description Of Adenoviral Vectors Containing a Fiber With the S408E,P409A (KO1) CAR-binding Mutation With and Without the cRGD Targeting Moiety

Two recombinant adenoviral vectors were prepared that contain the KO1 fiber mutation and are designated Av3nBgFKO1 and Av1nBgKO1RGD. These vectors contain the KO1 fiber mutation alone or in combination with a cRGD targeting moiety. The construction of these vectors is described below.

Genetic incorporation of the KO1 fiber mutation in combination with the cRGD targeting moiety to generate Av1nBgKO1RGD. All amino acid changes were incorporated into the fiber gene using the p5FloxPRGD adenoviral shuttle plasmid as the template as previously described in Example 2. This shuttle plasmid encodes a fiber that contains a cRGD peptide sequence, HCDCRGDCFC, inserted in the fiber HI loop. The cRGD peptide has been shown to bind to αv-integrins on the cell surface. Amino acid residues 408 and 409 in the AB loop of the fiber gene were changed using the Quickchange site-directed mutagenesis system (Stratagene, La Jolla CA). Substitution of these residues has been shown using the transient transfection/infection system to dramatically inhibit transduction of HeLa cells which express the adenoviral receptor, CAR. The resulting shuttle plasmid was called pSKO1 (Figure 8). The mutagenized fiber gene and the cRGD targeting moiety were incorporated into the adenoviral DNA backbone by cre-lox recombination. To do this, the pSKO1 shuttle plasmid (Figure 8) and the pAv1hlpr plasmid (Figure 4) were co-transfected with pCRE (described in Example 2) into 633 cells, a cell line that expresses the wildtype fiber {Von Seggern et al. 2000}. Expression of the CRE recombinase from pCRE mediates recombination between the lox sites in pSKO1 and in pAv1hlpr, resulting in full length Av1 viral DNA with the nuclear β-gal transgene in the E1 region to generate Av1nBgKO1RGD. This virus was initially propagated on 633 cells. After growth on these cells, the virus capsid contained both wildtype and mutant fiber proteins. To obtain viral particles containing only the modified fiber with the KO1 mutation and the RGD moiety, the viral preparation was used to infect AE1-2a cells {Gorziglia, Kadan, et al., 1996}, which do not express fiber.

Genetic incorporation of the KO1 fiber mutation into the adenoviral genome. The KO1 mutation alone was incorporated genetically into the adenoviral genome to generate Av3nBgFKO1. The KO1 mutation was cloned into a plasmid containing the full-length Av3 adenoviral genome {Gorzigliz, Kadan, et al., 1996} to generate pFLAv3nBgFKO1 (Figure 9). Transfections were carried out in 633 cells, and in this fiber complementing cell line, the

resulting viral DNA containing the KO1 mutation is capable of being packaged into infectious viral particles containing a mixture of wildtype fiber and mutant fiber proteins. Upon observation of CPE, the virus was purified by standard CsCl centrifugation procedures. In order to obtain viral particles containing only the adenoviral encoded mutated KO1 fiber protein with the S408E, P409A mutations, this viral preparation was used to infect AE1-2a cells, which do not express fiber. Viral particles were purified as described above.

In vitro evaluation of adenoviral vectors containing the KO1 fiber mutation. Several recombinant adenoviral vectors were used in these studies to demonstrate the function and specificity of the KO1 fiber mutation and the cRGD targeting moiety. These vectors are described in Table 3.

Table 3. Description of recombinant adenoviral vectors used

Vector	Description
Av3nBg	An E1, E2a, E3-deleted adenoviral vector encoding a nuclear localizing β-galactosidase
Av3nBgFKO1	The same as Av3nBg but containing the KO1 mutation in the fiber gene
Av15FHIRGD	An E1, E3-deleted vector encoding a nuclear localizing β-galactosidase and containing a cRGD ligand in the HI loop of fiber
Av1nBgKO1RGD	An E1, E3-deleted vector encoding a nuclear localizing β-galactosidase and containing both the KO1 fiber mutation and a cRGD ligand in the HI loop.

Transduction efficiency of adenoviral vectors containing AB loop mutations. As shown previously in example 1, adenoviruses pseudotyped with fiber proteins containing the S408E, P409A substitutions in the AB loop are severely affected in their ability to mediate gene transfer in HeLa cells, a CAR-expressing cell line. In order to demonstrate the function and specificity of the KO1 mutation and the ability to restore efficient gene transfer by using alternative ligand/receptor interactions, we tested the ability of the KO1-containing recombinant vectors to transduce various cell types. The four vectors listed in Table 3 were compared for transduction efficiency on HeLa cells, human diploid fibroblasts (HDFs), two different human hepatocellular carcinoma cell lines, Hep3Bs and HepG2s, and a mouse hepatocyte cell line, FL83b. The cells were seeded into the wells of a 24-well dish at 1-2 x 10^5 cells per well. The next day, the exact number of cells per well was determined for each cell line by counting a representative well for each cell type. The cells were transduced with various numbers of particles per cell (PPC), in triplicate, using each of the four vectors. Twenty-four hours after transduction, the cells were stained with X-gal and the percentage of β-galactosidase expressing cells was determined by counting cells under the microscope. Blue cells were counted in six different fields for each well. The total number of cells per field was determined by counting all cells in three fields from only one well, assuming that the total number of cells per field was the same for a given cell type across all wells. The

results showed that transduction of HeLa, Hep3B, HepG2, and FL83b cells by Av3nBgFKO1 was dramatically reduced compared to Av3nBg (Figures 10 and 11). HDFs, which express little or no CAR on their surface, were poorly transduced by both Av3nBg and Av3nBgFKO1 (Figure 10B). As expected, efficient transduction of HDFs was observed using the two vectors containing a cRGD ligand in the HI loop, Av15FHIRGD and Av1nBgKO1RGD (Figure 10B).

To determine whether the transduction observed in the above study was mediated by fiber-CAR interaction, competition binding experiments using HeLa cells were carried out using either full-length fiber protein or fiber knob as protein competitors (Figure 12). Competition was carried out by adding 40 μg/ml of the full-length fiber or 16 μg/ml of the fiber knob directly to the cells for 5 minutes at room temperature prior to infecting with vector. The results showed that either full-length fiber or fiber knob could efficiently block transduction of cells by Av3nBg. However, the low level of transduction of cells by Av3nBgFKO1 was not blocked by full-length fiber or fiber knob. This result shows that the low level of transduction by Av3nBgFKO1 was not mediated by fiber-CAR interaction. Similar results were also obtained on human hepatocytes. Fiber competition of Av3nBgFKO1 on mouse hepatocytes was not evaluated since transduction in the absence of competition was barely detectable.

<u>In vivo analysis of adenoviral vectors containing the KO1 fiber mutation</u>. Efficient in vivo targeting of adenoviral vectors requires both ablation of the normal tropism and the addition of a new tropism. We generated adenoviral vectors which were designed to achieve both of those requirements. (See Table 3.) To ablate the normal tropism, the adenovirus fiber protein was genetically modified to knockout its interaction with the coxsackie-adenovirus receptor (CAR). To retarget the vector to a different receptor, a cRGD targeting moiety was inserted into the HI loop of the fiber knob. In vitro studies demonstrate that adenoviral vectors containing mutations in the fiber knob which knockout its ability to interact with CAR are unable to transduce target cells. One particular fiber knockout mutant, called KO1, reduces transduction of HeLa cells to 1.4 % of the transduction level observed using a vector containing a wild-type fiber. Furthermore, in vitro data has been generated showing that cRGD ligand insertion into the HI loop of the fiber KO1 mutant restores transduction to levels equivalent to that seen with "wild-type' vector. These vectors were next analyzed in vivo using C57BL/6 male mice to assess the extent of liver detargeting and to determine the

tissue distribution of such vectors following tail vein administration and to determine whether insertion of the cRGD targeting ligand into the HI loop restores liver transduction.

In vivo study design. The study included 5 cohorts of 5 mice each. Adenoviral vectors encoding nuclear targeted β-galactosidase (nBg) were administered by tail vein injection. The dose was 1 x 10^{13} particles per kilogram. Mice were sacrificed 3 days after vector administration. Tissues, including liver, lung, heart, kidney, and spleen, were collected. Several assays were utilized to assess the efficiency of liver transduction and the vector biodistribution and included hexon PCR analysis, β-gal immunohistochemistry, and the β-gal Tropix assay. One group of mice received Hanks Balanced Salt Solution (HBSS) instead of adenoviral vector and served as a negative control. A second cohort received Av3nBg, which contains a "wild-type" fiber protein and served as a positive control. A third group received Av3nBgFKO1, a fourth group received Av1nBgKO1HIcRGD, and a fifth group received Av1nBgHIcRGD.

Vector Preparation. Each vector was diluted into sterile HBSS, at a final concentration of 1 x 10^{12} particles/ml. Mice in-groups 2 through 6 were injected with a volume of 10 ml/kg to achieve a vector dose of 1 x 10^{13} particles per kg. The HBSS control group received an equivalent dose volume.

Technical Methods. Administration of control and test articles was by bolus tail vein injection. Dose was determined from body weights obtained the day of administration. Animals were sacrificed approximately 72 hours after vector administration by carbon dioxide asphyxiation. Liver, heart, lung, spleen, and kidney were collected from each animal. Slices of each tissue approximately 2-3 mm thick were placed in neutral buffered formalin to preserve the sample for β-galactosidase immunohistochemistry. For optimal histology, one piece of each liver lobe, a lobe of the lung, a whole kidney, an end cross sectional piece of the spleen, and half of the heart cut longitudinally, were placed into the same container of neutral buffered formalin. The remaining tissue from each organ was placed into a 1 ml cryovial and frozen in dry ice to preserve it for hexon PCR analysis to determine vector content. For the liver, pieces of each lobe were frozen in dry ice to preserve it for hexon PCR analysis and other pieces of each lobe were placed in a "Tropix" vial, and frozen on dry ice.

The results of the immunohistochemical staining for β-galactosidase expression showed that Av3nBg, Av3nBgFKO1, Av15FHIRGD, and Av1nBgKO1RGD all yielded efficient transduction of hepatocytes. Av3nBgFKO1 yielded a higher percentage of β-

galactosidase expressing cells and a more intense staining than Av3nBg. This result was completely unexpected since Av3nBgFKO1 transduction of various cells in culture was dramatically reduced. Evaluation of β-galactosidase expression in mouse livers by a chemiluminescent assay (Figure 13) confirmed the results of the immunohistochemical staining. Mice that received Av3nBgFKO1 demonstrated higher levels of expression than those that received Av3nBg. A measurement of the vector content in hepatocytes was determined by a semi-quantitative hexon PCR assay (Figure 14). The results were consistent with both the immunohistochemical staining and the chemiluminescent assay. The vector content in hepatocytes was approximately 35% higher in the mice that received Av3nBgFKO1 than in those that received Av3nBg.

Summary. The fiber AB loop mutation contained in Av3nBgFKO1 ablates interaction with human and mouse CAR in vitro. However, in vivo this fiber AB loop mutation behaves quite unexpectantly as it was found to dramatically enhance adenoviral-mediated gene transfer to liver and other organs and results in increasing vector potency. This fiber modification will be useful for in vivo gene therapy strategies and will allow for lower doses of adenoviral vectors to be used systemically.

Example 4
Increased Liver Transduction Using Av3nBgFKO1 In Three Different Mouse Strains

The following experiment was done to determine whether the increased liver transduction observed with Av3nBgFKO1 compared to that using Av3nBg could be reproduced in various mouse strains.

In vivo analysis of adenoviral vectors containing the KO1 fiber mutation. C57BL/6, Balbc, and CD1 male mice were purchased from Harlan Sprague Dawley (Indianapolis, IN). When the mice were 5 weeks of age they received either HBSS (vehicle control), Av3nBg, or Av3nBgFKO1 via tail vein injection at a dose of 1×10^{13} particles per kg which is approximately 2×10^{11} particles per mouse. Cohorts of five mice received each treatment. The vector was diluted to 1×10^{12} particles per ml using Hanks Balanced Salt Solution (HBSS) immediately prior to injection. Three days after vector delivery, the animals were sacrificed and tissues including liver, lung, heart, kidney, and spleen were collected.

Technical methods for Galacto-Light Plus™ chemiluminescent assay. These tissue samples were used to analyze β-galactosidase expression using the Galacto-Light Plus™ chemiluminescent assay (Tropix, Inc., Foster City, CA) systems. Tissue samples were

collected in lysis matrix tubes containing two ceramic spheres (Bio101, Carlsbad, CA) and frozen on dry ice. The tissues were thawed and 500 µl of lysis buffer from the Galacto-Light Plus kit was added to each tube. The tissue was homogenized for 30 seconds using a FastPrep System (Bio101, Carlsbad, CA). Liver samples were homogenized for an additional 30 seconds. The β-galactosidase activity in tissue lysates was assayed according to the manufacturer's instructions.

Results. The results (Figure 15) showed that, on average, Av3nBgFKO1 yielded higher levels of liver transduction than Av3nBg in all three mouse strains.

Technical methods for β-galactosidase immunohistochemistry. In addition, slices of each tissue approximately 2-3 mm thick were placed in 10% neutral buffered formalin. After fixation, these samples were embedded in paraffin, sectioned, and analyzed by immunohistochemistry for β-galactosidase expression. A 1:1200 dilution of a rabbit anti-β-galactosidase antibody (ICN Pharmaceuticals, Inc.; Costa Mesa, CA) was used in conjunction with a Vectastain ABC kit from Vector Laboratories, Inc. (Burlingame, CA) to visualize positive cells.

Results. The results showed that Av3nBgFKO1 yielded higher levels of hepatocyte transduction than Av3nBg in all three mouse strains.

Example 5

Av3nBgFKO1 Transduced Primary Mouse Hepatocytes In Culture Relatively Poorly Compared To Av3nBg

The transduction efficiencies of Av3nBg and Av3nBgFKO1 were evaluated on primary mouse hepatocytes.

Isolation and culturing of primary murine hepatocytes. Primary murine hepatocytes were isolated from adult, male CD-1 mice following a two-step collagenase perfusion via the portal vein, modified from published procedures (Seglen, Methods Cell Biol 1973, 13:29-83; Liddle et al., J Gastro Hepatol 1998, 13:855-858; Marc et al., Eur J Biochem 2000, 267:963-970). The liver was perfused in situ with Liver Perfusion Medium (Life Technologies, Gaithersburg, MD) followed by treatment with Liver Digest Medium (collagenase-dispase; Life Technologies). The liver was minced, and cells were washed and centrifuged three times in Hepatocyte Wash medium (enriched William's E; Life Technologies) before being resuspended in Hepatocye Attachment Medium (Modified William's E, supplemented with

1% pen-strep and 5% FBS; Life Technologies). Viability was assessed by trypan blue exclusion. Cells were plated at approximately 1 x 10^5 viable cells per well on collagen type I-coated 24-well plates and allowed to attach for 2 hr at 37°C in 5% CO2. After 2 hr, unattached cells and media were removed, cells were washed and cultured in HepatoZYME-SFM (Life Technologies). Immunohistochemical staining for albumin confirmed the identity of these cells as hepatocytes.

Transduction efficiency of Av3nBgFKO1 on primary murine hepatocytes. Approximately 24 hours after plating, the cells were transduced with the adenoviral vectors Av3nBg and Av3nBgFKO1 at various numbers of particles per cell, ranging from 0 to 12,500. The cells were incubated with adenoviral vector for 1 hour at 37° C in a total volume of 0.2 ml of culture medium. Next, the cell monolayers were washed once with PBS, then 1 ml of the appropriate culture medium was added to each well. The cells were incubated for 24 hours to allow for β-galactosidase expression. The cell monolayers were then fixed and stained with X-Gal for 24 hours. The percentage of transduction was determined by light microscopy by counting the number of transduced, blue cells per total cells in a high-power field with a Nikon CK1 microscope; three fields were counted per well. Each vector dose was carried out in triplicate and the average percentage of transduction per high-power field (n=3 wells) was determined. The mean percent transduction obtained from at least three independent experiments was determined.

Results. The results (Figure 16) showed dramatically reduced transduction of primary mouse hepatocytes using Av3nBgFKO1 compared to Av3nBg.

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The disclosures of all patents, patent applications, publications (including published patent applications), depository accession numbers, and database accession numbers referred to in this specification are specifically incorporated herein by reference in their entirety to the same extent as if each such individual patent, patent application, publication, depository accession number, and database accession number were specifically and individually indicated to be incorporated in its entirety.

CLAIMS:

1. A mutated adenoviral fiber protein wherein at least one amino acid in the CD loop of a wild-type fiber protein of an adenovirus from subgroup C, D, or E, or the long wild-type fiber of an adenovirus from subgroup F, has been mutated to reduce or substantially eliminate the ability of said fiber protein to bind to the coxsackievirus-adenovirus receptor (CAR).
2. The mutated adenoviral fiber protein of claim 1, wherein said mutation substantially eliminates the ability of said protein to bind to said CAR.
3. The mutated adenoviral fiber protein of claim 2, wherein said fiber protein is an adenovirus serotype 5 fiber protein.
4. The mutated adenoviral fiber protein of claim 3, wherein said fiber protein contains at least one mutation at amino acid positions 441 and 442 of the wild-type fiber protein.
5. The mutated adenoviral fiber protein of claim 4, wherein said fiber protein further comprises a mutation at one or more of the following amino acid positions of the wild-type fiber protein: 408, 409, 460, 509, 510, 538, and 539.
6. The mutated adenoviral fiber protein of claim 4, wherein said fiber protein further comprises at least one mutation at amino acid positions 408 and 409 of the wild-type fiber protein.
7. A mutated adenovirus serotype 5 fiber protein wherein said fiber protein comprises a mutation at one or more of the following amino acid positions of the wild-type fiber protein: 460, 509, 510, 538, and 539, wherein said mutation reduces or substantially eliminates the ability of said fiber protein to bind to CAR.
8. A polynucleotide encoding the protein of claim 1.
9. A polynucleotide encoding the protein of claim 3.
10. A polynucleotide encoding the protein of claim 4.
11. A polynucleotide encoding the protein of claim 5.
12. A polynucleotide encoding the protein of claim 7.
13. An adenoviral particle comprising the fiber protein of claim 1.
14. An adenoviral particle comprising the fiber protein of claim 3.
15. An adenoviral particle comprising the fiber protein of claim 4.
16. An adenoviral particle comprising the fiber protein of claim 5.
17. An adenoviral particle comprising the fiber protein of claim 7.
18. The adenoviral particle of claim 13 further comprising a targeting ligand included in a capsid protein of said particle.

19. The adenoviral particles of claim 18 wherein said capsid protein is the mutated adenoviral fiber protein.
20. The adenoviral particle of claim 19 further comprising at least one heterologous polynucleotide.
21. The adenoviral particle of any one of claims 14-17 further comprising a targeting ligand included in a capsid protein of said particle.
22. The adenoviral particle of claim 21 wherein said capsid protein is the mutated adenoviral fiber protein.
23. The adenoviral particle of claim 22 further comprising at least one heterologous polynucleotide.
24. An adenovirus packaging cell comprising the polynucleotide of claim 8.
25. A method of making the adenoviral particle of claim 13, comprising the steps of:
    transferring the adenovirus genome to be packaged in said particle into the packaging cell of claim 24;
    culturing said packaging cell; and
    recovering an adenoviral particle produced by said cell.
26. A method of making the adenoviral particle of claim 18 comprising the steps of:
    transferring the adenovirus genome to be packaged in said particle into a cell having adenovirus polynucleotides that provide proteins necessary for the replication, maturation, and packaging of said genome;
    culturing said cell under conditions permitting the production of said particle; and
    recovering an adenoviral particle produced by said cell.
27. A method of expressing a heterologous polynucleotide in a cell comprising infecting said cell with the adenoviral particle of claim 20.
28. The method of claim 27, wherein said cell is a mammalian cell.
29. The method of claim 28, wherein said mammalian cell is a primate cell.
30. The method of claim 29, wherein said primate cell is a human cell.
31. A composition comprising the adenoviral particle of claim 18 in a pharmaceutically acceptable carrier.
32. A composition comprising the adenoviral particle of claim 20 in a pharmaceutically acceptable carrier.
33. A mutated adenovirus serotype 5 fiber protein wherein said fiber protein contains mutations at amino acid positions 408 and 409 of the wild-type fiber protein.

34. The mutated fiber protein of claim 33, wherein said protein contains deletions at amino acid positions 408 and 409 of the wild-type fiber protein.

35. The mutated fiber protein of claim 33, wherein said protein contains amino acid substitutions at amino acid positions 408 and 409 of the wild-type fiber protein.

36. The mutated fiber protein of claim 35, wherein glutamic acid is substituted for serine at position 408 and alanine is substituted for proline at position 409 (SEQ ID NO: 4).

37. A polynucleotide encoding the protein of claims 33-36.

38. An adenoviral particle comprising the fiber protein of claims 33-36.

39. The adenoviral particle of claim 38, further comprising a targeting ligand included in a capsid protein of said particle.

40. The adenoviral particle of claim 39, further comprising at least one heterologous polynucleotide.

41. The adenoviral particle of claim 38, wherein at least one of the penton proteins of said particle has been modified to delete the RGD sequence.

42. An adenovirus packaging cell comprising the polynucleotide of claim 37.

43. A method of making the adenoviral particle of claim 38, comprising the steps of:
    transferring the adenovirus genome to be packaged in said particle into the packaging cell of claim 42;
    culturing said packaging cell; and
    recovering an adenoviral particle produced by said cell.

44. A method of making the adenoviral particle of claim 38, comprising the steps of:
    transferring the adenovirus genome to be packaged in said particle into a cell having adenovirus polynucleotides that provide proteins necessary for the replication, maturation, and packaging of said genome;
    culturing said cell under conditions permitting the production of said particle; and
    recovering an adenoviral particle produced by said cell.

45. A method of expressing a heterologous polynucleotide in a cell comprising infecting said cell with the adenoviral particle of claim 40.

46. The method of claim 45, wherein said cell is a mammalian cell.

47. The method of claim 45, wherein said cell is a primate cell.

48. The method of claim 45, wherein said cell is a human cell.

49. A composition comprising the adenoviral particle of claim 40 in a pharmaceutically acceptable carrier.

50. A method of enhancing adenoviral-mediated gene transfer to and expression in hepatocytes comprising the steps of administering adenoviral particles of claim 40 to said hepatocytes.

51. A method of enhancing adenoviral-mediated gene transfer to and expression in hepatocytes comprising the steps of:

preparing an adenovirus particle comprising a mutated adenovirus serotype 5 fiber protein, wherein glutamic acid is substituted for serine at amino acid position 408 and alanine is substituted for proline at amino acid position 409, and further comprising a heterologous gene; and infecting hepatocytes with said adenovirus particle.

52. A method of expressing a protein in a mammal comprising the step of administering the adenoviral particle of claim 20 or claim 40 to said mammal, wherein said particle transduces a cell in said mammal and said heterologous polynucleotide expresses said protein in said cell.

53. The method of claim 52, wherein said mammal is a primate.

54. The method of claim 53, wherein said primate is a human.

55. A method of expressing a protein in the liver of a mammal comprising administering a sufficient amount of the adenoviral particles of claim 40 for said particles to transduce cells in the liver of said mammal.

56. The method of claim 55, wherein said amount comprises approximately 1 particle per kilogram of body weight to approximately 10^{13} particles per kilogram of body weight.

57. The method of claim 55, wherein said amount comprises approximately 10^4 particles per kilogram of body weight to approximately 10^{12} particles per kilogram of body weight.

58. The method of claim 55, wherein said amount comprises approximately 10^8 particles per kilogram of body weight to approximately 10^{11} particles per kilogram of body weight.

59. An adenoviral vector comprising the polynucleotide of any one of claims 8-12.

60. An adenoviral vector comprising the polynucleotide of claim 37.

61. The adenoviral particle of claims 13, 18, 20, 38, 39, 40, or 41, wherein said adenoviral particle is a replication conditional adenovirus.

62. The adenoviral particle of claim 61, wherein said adenovirus is an oncolytic adenovirus.

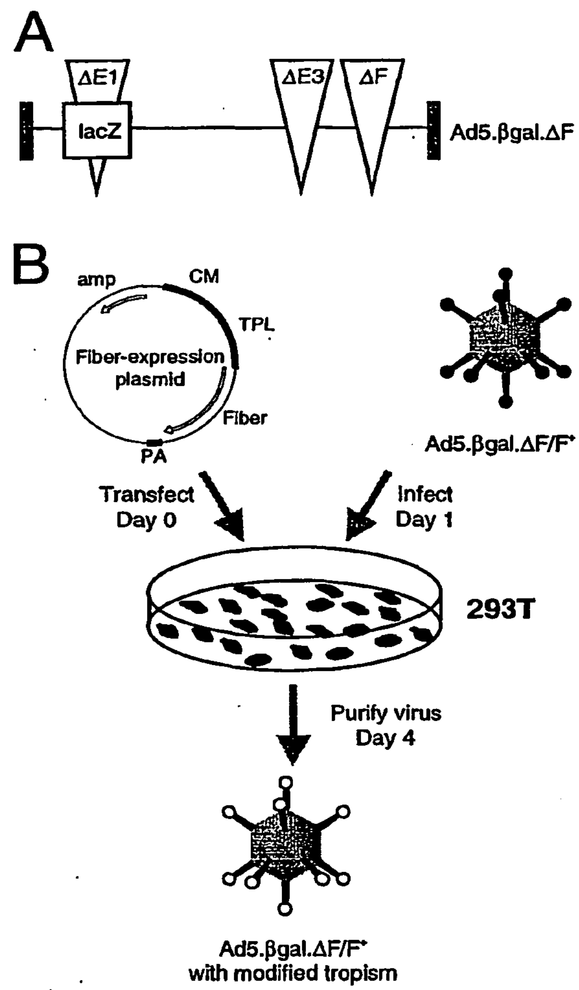

A
ΔE1
lacZ
ΔE3
ΔF
Ad5.βgal.ΔF
B
amp
CM
TPL
Fiber-expression plasmid
Fiber
PA
Ad5.βgal.ΔF/F+
Transfect Day 0
Infect Day 1
293T
Purify virus Day 4
Ad5.βgal.ΔF/F*
with modified tropism

Figure 1

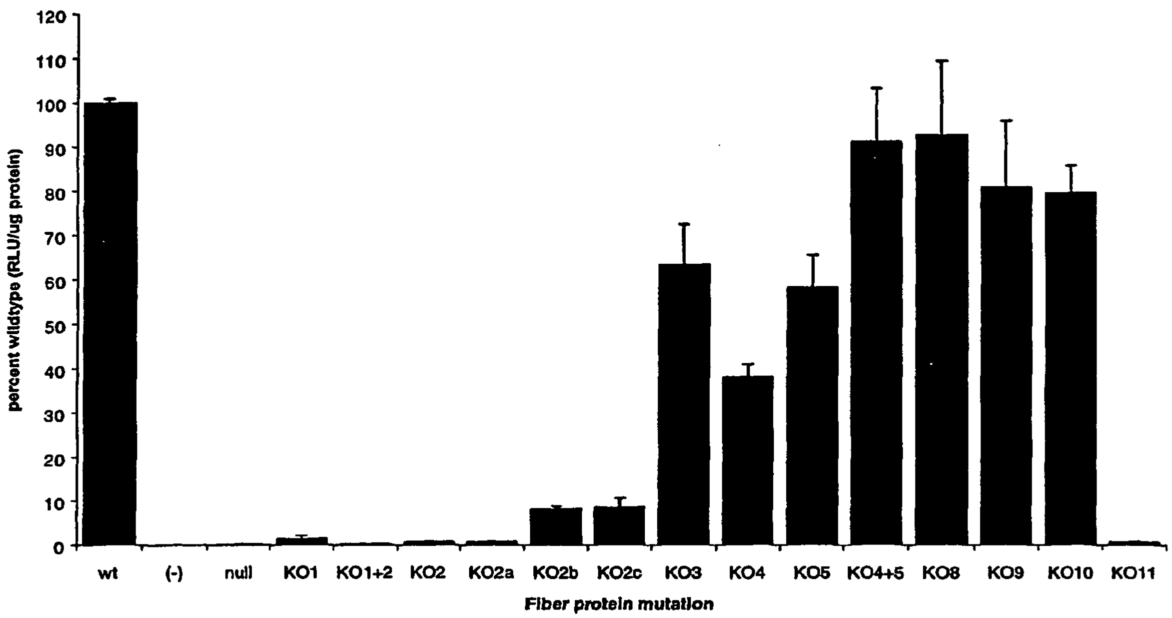
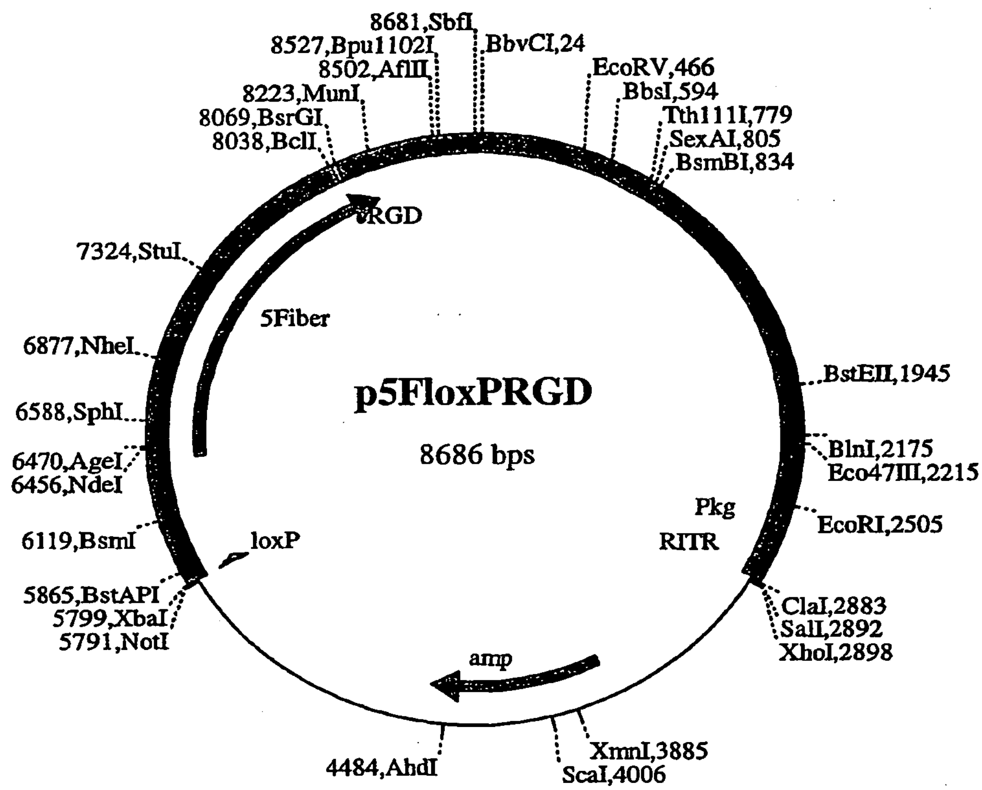

120
110
100
90
80
70
60
50
40
30
20
10
0
percent wildtype (RLU/ug protein)
wt
(-)
null
KO1
KO1+2
KO2
KO2a
KO2b
KO2c
KO3
KO4
KO5
KO4+5
KO8
KO9
KO10
KO11
Fiber protein mutation

Figure 2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BbsI,594
Tth111I,779
SexAI,805
BsmBI,834
RGD
7324,StuI
5Fiber
6877,NheI
p5FloxPRGD
8686 bps
BstEII,1945
6588,SphI
6470,AgeI
6456,NdeI
BlnI,2175
Eco47III,2215
Pkg
EcoRI,2505
6119,BsmI
loxP
RITR
5865,BstAPI
5799,XbaI
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SalI,2892
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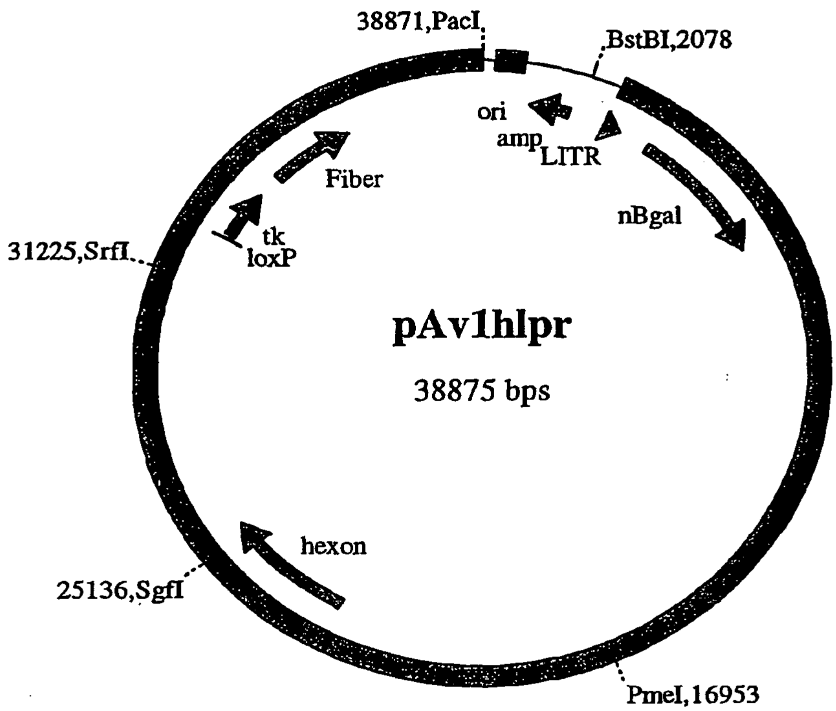
amp
XmnI,3885
ScaI,4006
4484,AhdI

Figure 3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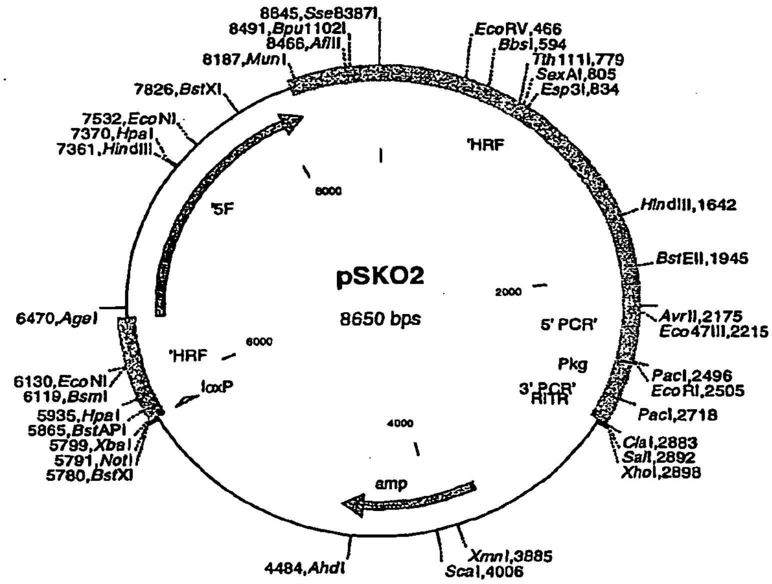
PmeI,16953

Figure 4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8187,MunI
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7532,EcoNI
7370,HpaI
7361,HindIII
6470,AgeI
6130,EcoNI
6119,BsmI
5935,HpaI
5865,BstAPI
5799,XbaI
5791,NotI
5780,BstXI
4484,AhdI
EcoRV,466
BbsI,594
Tth111I,779
SexAI,805
Esp3I,834
HindIII,1642
BstEII,1945
AvrII,2175
Eco47III,2215
PacI,2496
EcoRI,2505
PacI,2718
ClaI,2883
SalI,2892
XhoI,2898
XmnI,3885
ScaI,4006
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'5F
'HRF
loxP
5' PCR'
Pkg
3' PCR'
RITR
amp
2000
4000
6000
8000

Figure 5

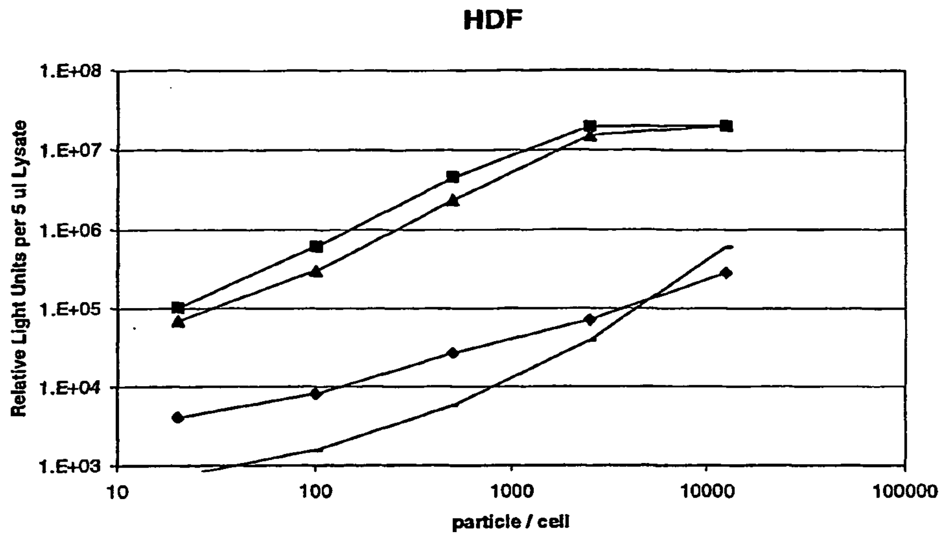


Figure 6A

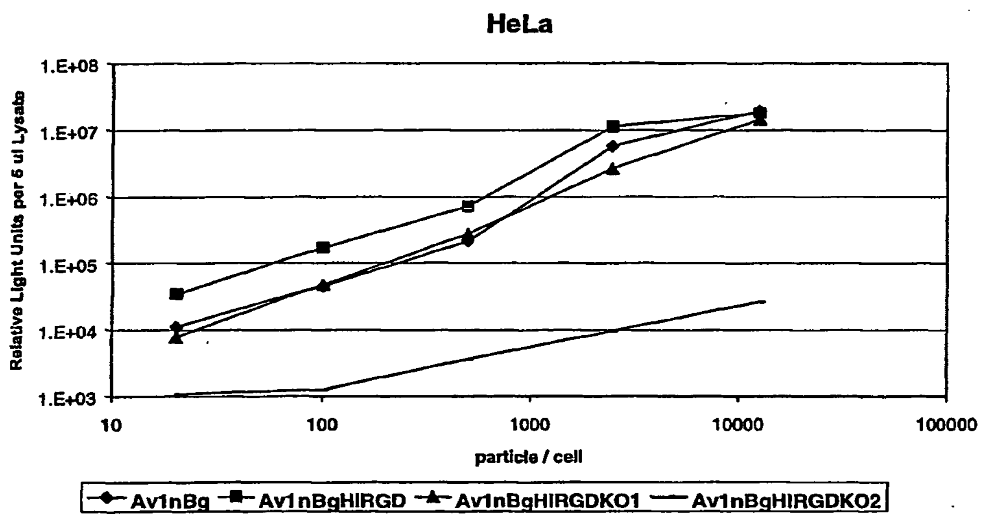


Figure 6B

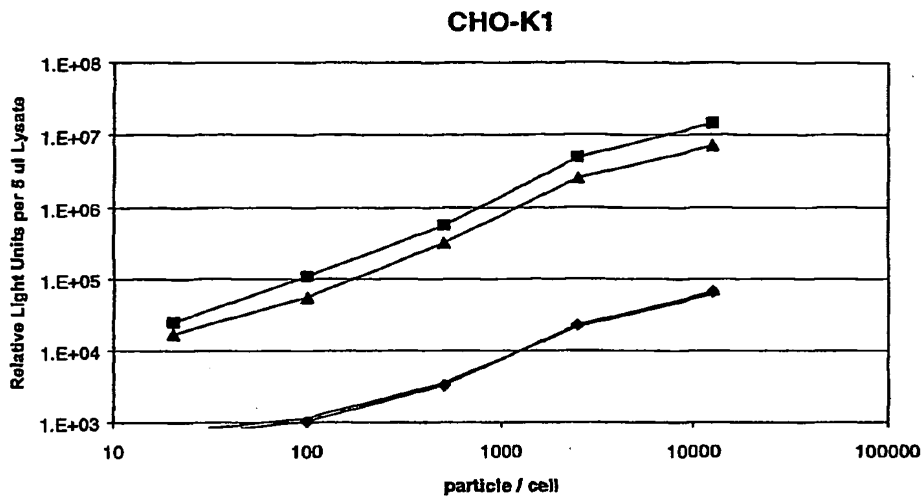


Figure 6C

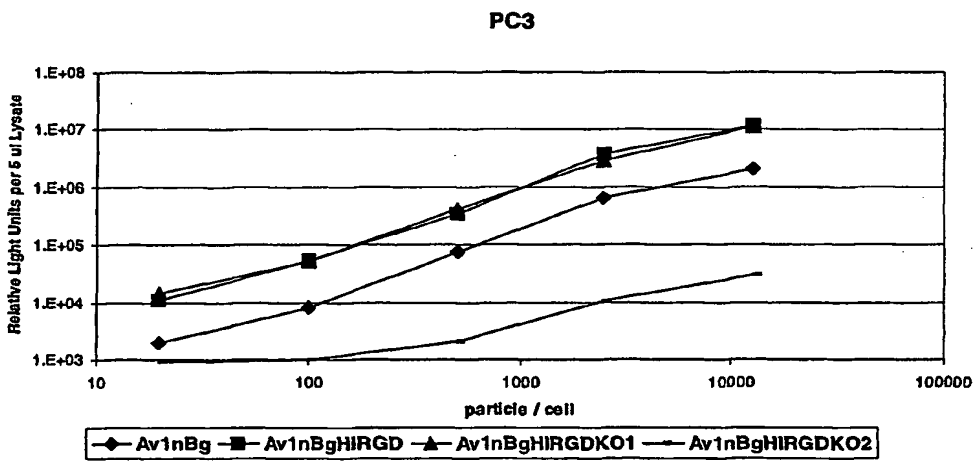
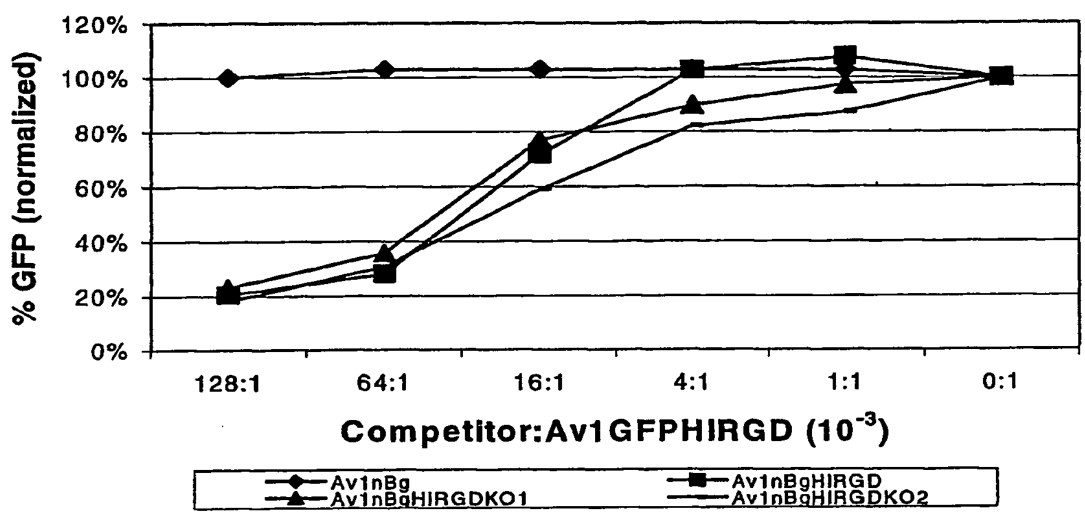


Figure 6D


120%
100%
80%
60%
40%
20%
0%
% GFP (normalized)
128:1
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16:1
4:1
1:1
0:1
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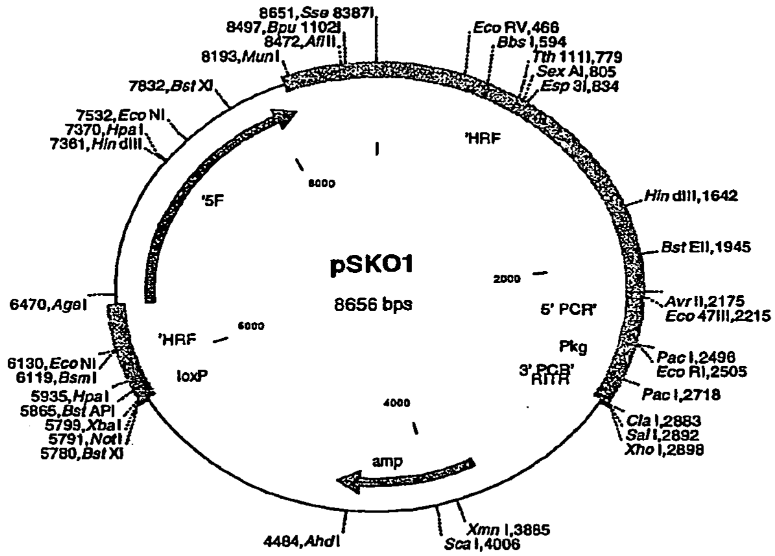
Av1nBgHIRGD
Av1nBgHIRGDKO1
Av1nBgHIRGDKO2

Figure 7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5935,HpaI
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ScaI,4006
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loxP
amp
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4000
6000
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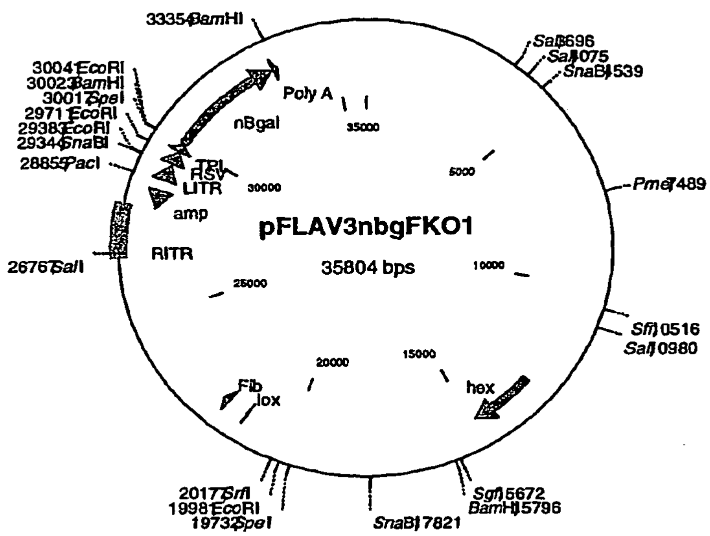
Figure 8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hex
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25000
20000
15000
10000
5000
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2971 EcoRI
2934 SnaBI
2885 PacI
2676 SalI
2017 SrfI
1998 EcoRI
1973 SpeI
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PmeI 7489
SnaBI 1539

Figure 9

Fig. 10A Transduction of HeLa Cells

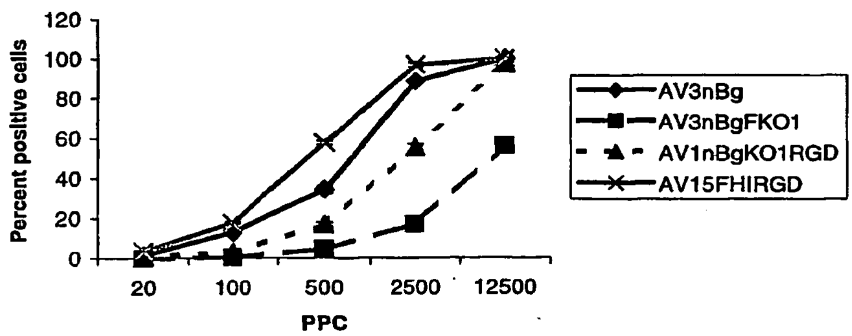


Fig. 10B Transduction on HDF cells

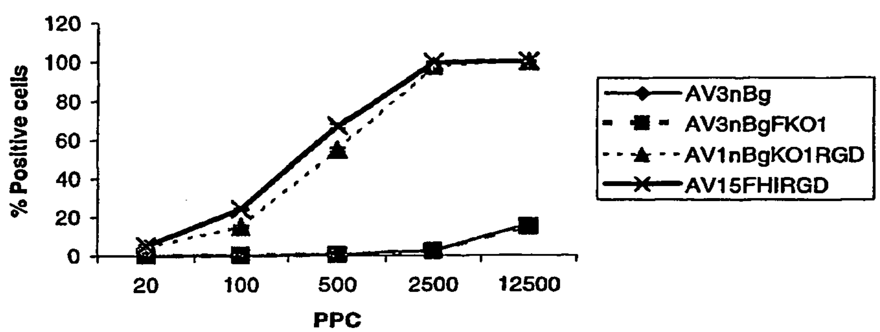


Fig. 11A Transduction on Human Hepatocellular Carcinoma, Hep3B cells

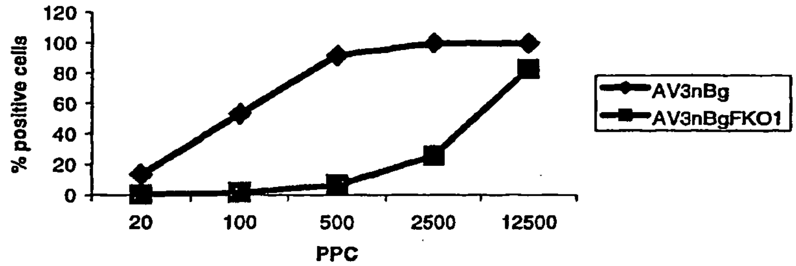


Fig. 11B Transduction on Human Hepatocellular Carcinoma, HepG2 cells

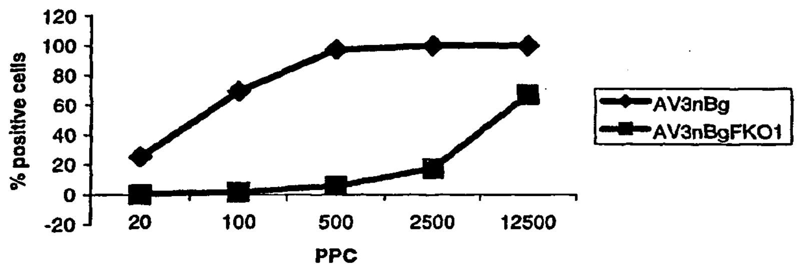
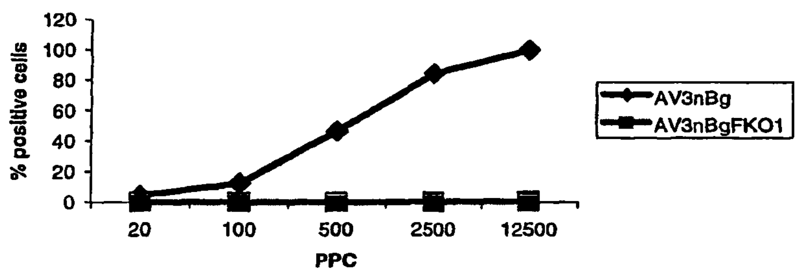


Fig. 11C Transduction on Mouse Hepatocytes, FL83b cells



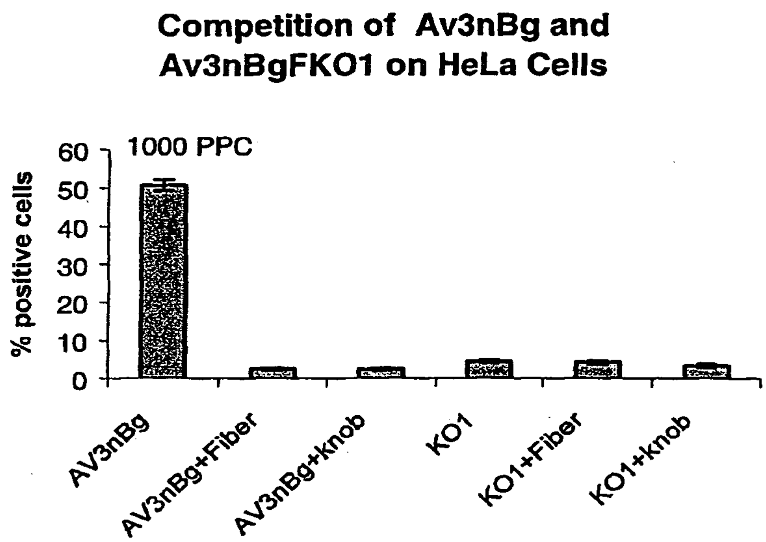

Competition of Av3nBg and
Av3nBgFKO1 on HeLa Cells
1000 PPC
% positive cells
60
50
40
30
20
10
0
AV3nBg
AV3nBg+Fiber
AV3nBg+knob
KO1
KO1+Fiber
KO1+knob

Figure 12

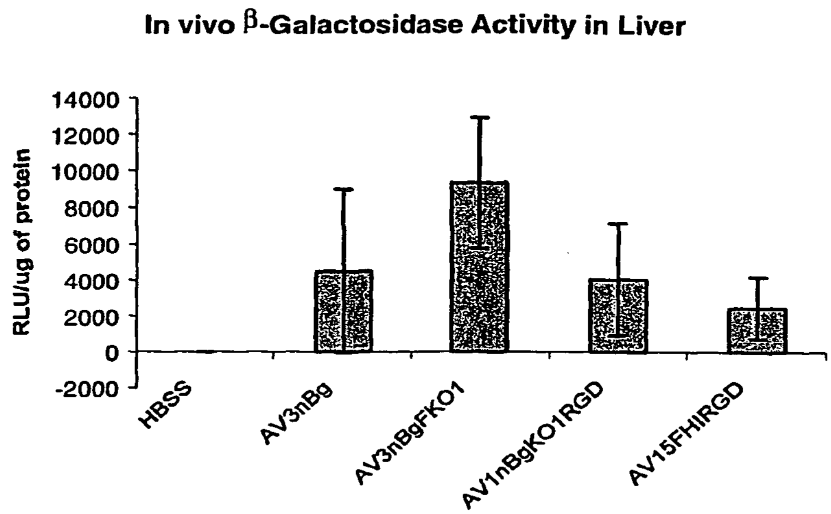

In vivo β-Galactosidase Activity in Liver
RLU/ug of protein
14000
12000
10000
8000
6000
4000
2000
0
-2000
HBSS
AV3nBg
AV3nBgFKO1
AV1nBgKO1RGD
AV15FHIRGD

Figure 13

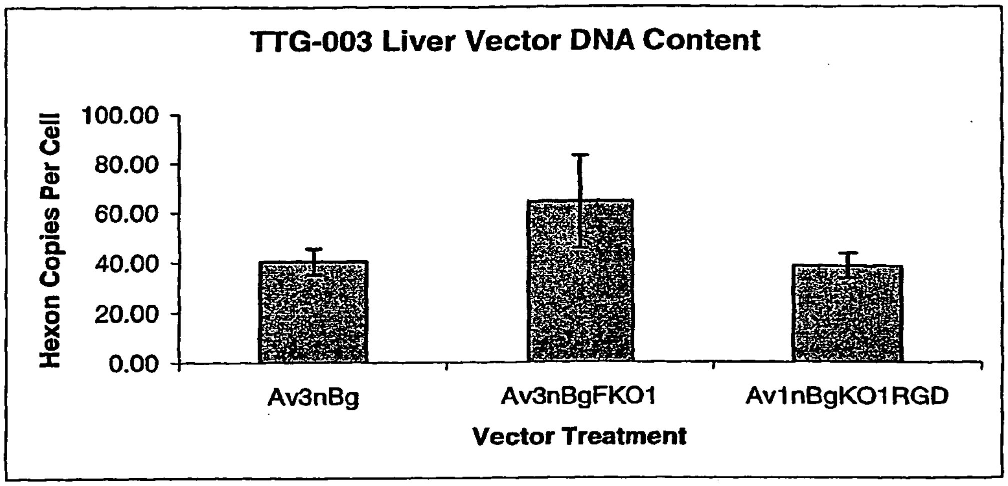
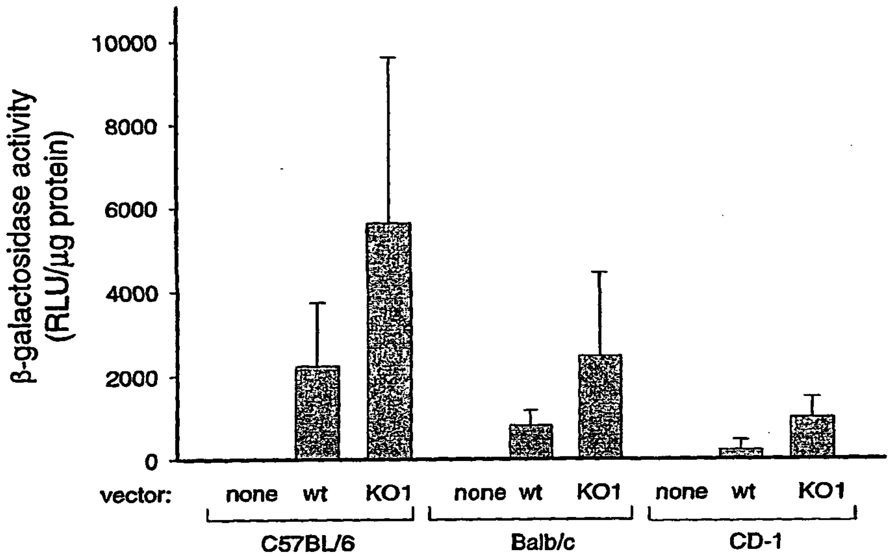

TTG-003 Liver Vector DNA Content
Hexon Copies Per Cell
100.00
80.00
60.00
40.00
20.00
0.00
Av3nBg
Av3nBgFKO1
Av1nBgKO1RGD
Vector Treatment

Figure 14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(RLU/µg protein)
10000
8000
6000
4000
2000
0
vector:
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wt
KO1
none
wt
KO1
none
wt
KO1
C57BL/6
Balb/c
CD-1

Figure 15

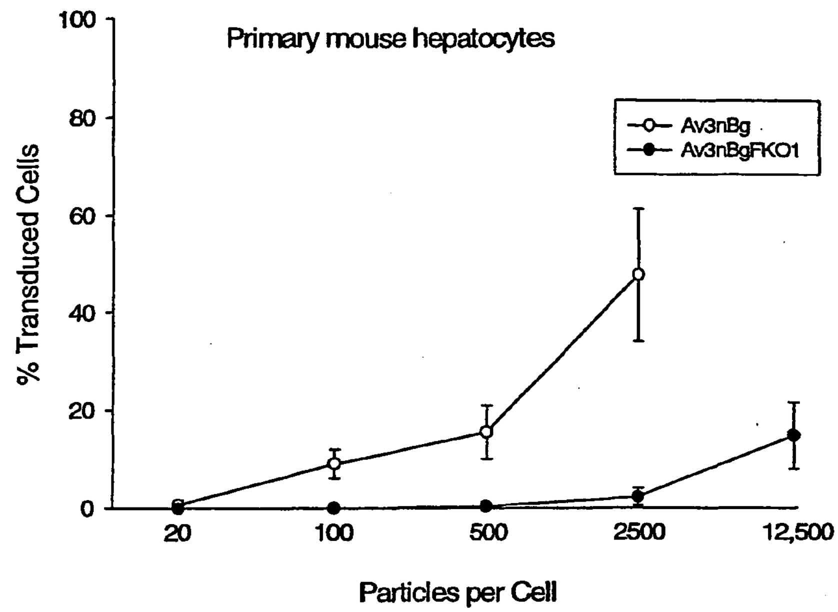
Primary mouse hepatocytes
% Transduced Cells
100
80
60
40
20
0
Av3nBg
Av3nBgFKO1
20
100
500
2500
12,500
Particles per Cell

Figure 16

SEQUENCE LISTING

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<160> 43

<170> PatentIn version 3.0

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<220>
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Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30

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Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

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Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

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Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac 288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta 336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100 105 110

act gtg gct gcc gcc gca cct cta atg gtc gcg ggc aac aca ctc acc 384
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
115 120 125

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130 135 140

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Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145 150 155 160

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Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165 170 175

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Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190

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Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205

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   720
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr

225                 230                 235                 240

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        275                 280                 285

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Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn

    290                 295                 300

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   960
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu

305                 310                 315                 320

gtt aac cta agc act gcc aag ggg ttg atg ttt gac gct aca gcc ata
  1008
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile

                325                 330                 335
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340 345 350

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1104
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365

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1152
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370 375 380

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1200
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385 390 395 400

ttg tgg acc aca cca gct cca tct cct aac tgt aga cta aat gca gag
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405 410 415

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1296
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
420 425 430

ctt gct aca gtt tca gtt ttg gct gtt aaa ggc agt ttg gct cca ata
1344
Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
435 440 445

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1392
Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
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485 490 495

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        35   40   45

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Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
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Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
                85   90   95

Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
            100   105   110

Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
        115   120   125

Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
    130   135   140

Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145   150   155   160

Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
                165   170   175

Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
            180   185   190

Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205

Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220

Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240

Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270

Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290 295 300

Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320

Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325 330 335

Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340 345 350

Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365

Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370 375 380

Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385 390 395 400

Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
405 410 415

```
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
            420                 425                 430

Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
        435                 440                 445

Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
    450                 455                 460

Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
465                 470                 475                 480

Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
                485                 490                 495

Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
            500                 505                 510

Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
        515                 520                 525

Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp
    530                 535                 540

Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly
545                 550                 555                 560

His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser
                565                 570                 575

Tyr Ile Ala Gln Glu
            580
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<210> 3
<211> 1746
<212> DNA
<213> Artificial

<220>
<223> Codes for a mutated Human Adenovirus type 5 fiber protein.

<220>
<221> CDS
<222> (1)..(1746)

<220>
<221> mutation
<222> (1222)..(1227)

<400> 3
atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca
48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc
96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30

ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct
144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc
192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc
240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac
288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta
336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100 105 110

act gtg gct gcc gcc gca cct cta atg gtc gcg ggc aac aca ctc acc

```
   384
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr

        115                 120                 125

atg caa tca cag gcc ccg cta acc gtg cac gac tcc aaa ctt agc att
   432
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile

    130                 135                 140

gcc acc caa gga ccc ctc aca gtg tca gaa gga aag cta gcc ctg caa
   480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln

145                 150                 155                 160

aca tca ggc ccc ctc acc acc acc gat agc agt acc ctt act atc act
   528
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr

                165                 170                 175

gcc tca ccc cct cta act act gcc act ggt agc ttg ggc att gac ttg
   576
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu

            180                 185                 190

aaa gag ccc att tat aca caa aat gga aaa cta gga cta aag tac ggg
   624
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly

        195                 200                 205

gct cct ttg cat gta aca gac gac cta aac act ttg acc gta gca act
   672
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr

    210                 215                 220

ggt cca ggt gtg act att aat aat act tcc ttg caa act aaa gtt act
   720
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr

225                 230                 235                 240

gga gcc ttg ggt ttt gat tca caa ggc aat atg caa ctt aat gta gca
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768
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala

245 250 255

gga gga cta agg att gat tct caa aac aga cgc ctt ata ctt gat gtt
816
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val

260 265 270

agt tat ccg ttt gat gct caa aac caa cta aat cta aga cta gga cag
864
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln

275 280 285

ggc cct ctt ttt ata aac tca gcc cac aac ttg gat att aac tac aac
912
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn

290 295 300

aaa ggc ctt tac ttg ttt aca gct tca aac aat tcc aaa aag ctt gag
960
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu

305 310 315 320

gtt aac cta agc act gcc aag ggg ttg atg ttt gac gct aca gcc ata
1008
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile

325 330 335

gcc att aat gca gga gat ggg ctt gaa ttt ggt tca cct aat gca cca
1056
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro

340 345 350

aac aca aat ccc ctc aaa aca aaa att ggc cat ggc cta gaa ttt gat
1104
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp

355 360 365

tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac

1152
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp

370 375 380

agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act
1200
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr

385 390 395 400

ttg tgg acc aca cca gct cca gag gct aac tgt aga cta aat gca gag
1248
Leu Trp Thr Thr Pro Ala Pro Glu Ala Asn Cys Arg Leu Asn Ala Glu

405 410 415

aaa gat gct aaa ctc act ttg gtc tta aca aaa tgt ggc agt caa ata
1296
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile

420 425 430

ctt gct aca gtt tca gtt ttg gct gtt aaa ggc agt ttg gct cca ata
1344
Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile

435 440 445

tct gga aca gtt caa agt gct cat ctt att ata aga ttt gac gaa aat
1392
Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn

450 455 460

gga gtg cta cta aac aat tcc ttc ctg gac cca gaa tat tgg aac ttt
1440
Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe

465 470 475 480

aga aat gga gat ctt act gaa ggc aca gcc tat aca aac gct gtt gga
1488
Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly

485 490 495

ttt atg cct aac cta tca gct tat cca aaa tct cac ggt aaa act gcc

1536
Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala

500 505 510

aaa agt aac att gtc agt caa gtt tac tta aac gga gac aaa act aaa
1584
Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys

515 520 525

cct gta aca cta acc att aca cta aac ggt aca cag gaa aca gga gac
1632
Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp

530 535 540

aca act cca agt gca tac tct atg tca ttt tca tgg gac tgg tct ggc
1680
Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly

545 550 555 560

cac aac tac att aat gaa ata ttt gcc aca tcc tct tac act ttt tca
1728
His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser

565 570 575

tac att gcc caa gaa taa
1746
Tyr Ile Ala Gln Glu

580

<210> 4
<211> 581
<212> PRT
<213> Artificial

<400> 4

Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100 105 110

Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
115 120 125

Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
130 135 140

Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145 150 155 160

Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165 170 175

Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190

Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205

Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220

Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240

Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

Gly	Gly	Leu	Arg	Ile	Asp	Ser	Gln	Asn	Arg	Arg	Leu	Ile	Leu	Asp	Val
			260					265					270		
Ser	Tyr	Pro	Phe	Asp	Ala	Gln	Asn	Gln	Leu	Asn	Leu	Arg	Leu	Gly	Gln
		275					280					285			
Gly	Pro	Leu	Phe	Ile	Asn	Ser	Ala	His	Asn	Leu	Asp	Ile	Asn	Tyr	Asn
	290					295					300				
Lys	Gly	Leu	Tyr	Leu	Phe	Thr	Ala	Ser	Asn	Asn	Ser	Lys	Lys	Leu	Glu
305					310					315					320
Val	Asn	Leu	Ser	Thr	Ala	Lys	Gly	Leu	Met	Phe	Asp	Ala	Thr	Ala	Ile
				325					330					335	
Ala	Ile	Asn	Ala	Gly	Asp	Gly	Leu	Glu	Phe	Gly	Ser	Pro	Asn	Ala	Pro
			340					345					350		
Asn	Thr	Asn	Pro	Leu	Lys	Thr	Lys	Ile	Gly	His	Gly	Leu	Glu	Phe	Asp
		355					360					365			
Ser	Asn	Lys	Ala	Met	Val	Pro	Lys	Leu	Gly	Thr	Gly	Leu	Ser	Phe	Asp
	370					375					380				
Ser	Thr	Gly	Ala	Ile	Thr	Val	Gly	Asn	Lys	Asn	Asn	Asp	Lys	Leu	Thr
385					390					395					400
Leu	Trp	Thr	Thr	Pro	Ala	Pro	Glu	Ala	Asn	Cys	Arg	Leu	Asn	Ala	Glu
				405					410					415	
Lys	Asp	Ala	Lys	Leu	Thr	Leu	Val	Leu	Thr	Lys	Cys	Gly	Ser	Gln	Ile
			420					425					430		
Leu	Ala	Thr	Val	Ser	Val	Leu	Ala	Val	Lys	Gly	Ser	Leu	Ala	Pro	Ile
		435					440					445			
Ser	Gly	Thr	Val	Gln	Ser	Ala	His	Leu	Ile	Ile	Arg	Phe	Asp	Glu	Asn
	450					455					460				
Gly	Val	Leu	Leu	Asn	Asn	Ser	Phe	Leu	Asp	Pro	Glu	Tyr	Trp	Asn	Phe
465					470					475					480

Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
485 490 495

Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
500 505 510

Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
515 520 525

Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp
530 535 540

Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly
545 550 555 560

His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser
565 570 575

Tyr Ile Ala Gln Glu
580

<210> 5
<211> 1740
<212> DNA
<213> Artificial

<220>
<223> Codes for a mutated Human Adenovirus type 5 fiber protein. Nucle
otides corresponding to amino acids 441(V) & 442(K) of wild-type
fiber were deleted

<220>
<221> CDS
<222> (1)..(1740)

<400> 5
atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca 48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc 96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro

20 25 30

ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct 144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc 192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc 240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac 288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta 336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100 105 110

act gtg gct gcc gcc gca cct cta atg gtc gcg ggc aac aca ctc acc 384
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
115 120 125

atg caa tca cag gcc ccg cta acc gtg cac gac tcc aaa ctt agc att 432
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
130 135 140

gcc acc caa gga ccc ctc aca gtg tca gaa gga aag cta gcc ctg caa 480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln

145 150 155 160

aca tca ggc ccc ctc acc acc acc gat agc agt acc ctt act atc act 528
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165 170 175

gcc tca ccc cct cta act act gcc act ggt agc ttg ggc att gac ttg 576
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190

aaa gag ccc att tat aca caa aat gga aaa cta gga cta aag tac ggg 624
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205

gct cct ttg cat gta aca gac gac cta aac act ttg acc gta gca act 672
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220

ggt cca ggt gtg act att aat aat act tcc ttg caa act aaa gtt act 720
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240

gga gcc ttg ggt ttt gat tca caa ggc aat atg caa ctt aat gta gca 768
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

gga gga cta agg att gat tct caa aac aga cgc ctt ata ctt gat gtt 816
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270

agt tat ccg ttt gat gct caa aac caa cta aat cta aga cta gga cag 864
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln

275 280 285

ggc cct ctt ttt ata aac tca gcc cac aac ttg gat att aac tac aac
912
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn

290 295 300

aaa ggc ctt tac ttg ttt aca gct tca aac aat tcc aaa aag ctt gag
960
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu

305 310 315 320

gtt aac cta agc act gcc aag ggg ttg atg ttt gac gct aca gcc ata
1008
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile

325 330 335

gcc att aat gca gga gat ggg ctt gaa ttt ggt tca cct aat gca cca
1056
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro

340 345 350

aac aca aat ccc ctc aaa aca aaa att ggc cat ggc cta gaa ttt gat
1104
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp

355 360 365

tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac
1152
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp

370 375 380

agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act
1200
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr

385 390 395 400

ttg tgg acc aca cca gct cca tct cct aac tgt aga cta aat gca gag
1248
Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu

405 410 415

aaa gat gct aaa ctc act ttg gtc tta aca aaa tgt ggc agt caa ata
1296
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile

420 425 430

ctt gct aca gtt tca gtt ttg gcc ggc agt ttg gct cca ata tct gga
1344
Leu Ala Thr Val Ser Val Leu Ala Gly Ser Leu Ala Pro Ile Ser Gly

435 440 445

aca gtt caa agt gct cat ctt att ata aga ttt gac gaa aat gga gtg
1392
Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn Gly Val

450 455 460

cta cta aac aat tcc ttc ctg gac cca gaa tat tgg aac ttt aga aat
1440
Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe Arg Asn

465 470 475 480

gga gat ctt act gaa ggc aca gcc tat aca aac gct gtt gga ttt atg
1488
Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe Met

485 490 495

cct aac cta tca gct tat cca aaa tct cac ggt aaa act gcc aaa agt
1536
Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala Lys Ser

500 505 510

aac att gtc agt caa gtt tac tta aac gga gac aaa act aaa cct gta
1584
Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys Pro Val

515 520 525

aca cta acc att aca cta aac ggt aca cag gaa aca gga gac aca act
1632
Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp Thr Thr

530 535 540

cca agt gca tac tct atg tca ttt tca tgg gac tgg tct ggc cac aac
1680
Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn

545 550 555 560

tac att aat gaa ata ttt gcc aca tcc tct tac act ttt tca tac att
1728
Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile

565 570 575

gcc caa gaa taa
1740
Ala Gln Glu

<210> 6
<211> 579
<212> PRT
<213> Artificial

<400> 6

Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100 105 110

Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
115 120 125

Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
130 135 140

Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145 150 155 160

Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165 170 175

Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190

Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205

Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220

Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240

Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270

Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290 295 300

Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320

Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325 330 335

Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340 345 350

Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365

Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370 375 380

Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385 390 395 400

Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
405 410 415

Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
420 425 430

Leu Ala Thr Val Ser Val Leu Ala Gly Ser Leu Ala Pro Ile Ser Gly
435 440 445

Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn Gly Val
450 455 460

Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe Arg Asn
465 470 475 480

Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe Met
485 490 495

Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala Lys Ser
500 505 510

Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys Pro Val
515 520 525

Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp Thr Thr
530 535 540

```
Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn
545                 550                 555                 560

Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile
                565                 570                 575

Ala Gln Glu


<210>  7
<211>  1740
<212>  DNA
<213>  Artificial

<220>
<223>  Codes for a mutated Human Adenovirus type 5 fiber protein. Combin
       ation of the mutant fiber encoded in Seq ID: #3 & 5

<220>
<221>  CDS
<222>  (1)..(1740)

<400>  7
atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca
  48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro

1               5                   10                  15

tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc
  96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro

            20                  25                  30

ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct
  144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser

        35                  40                  45

ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc
  192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu

    50                  55                  60
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aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc 240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac 288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta 336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100 105 110

act gtg gct gcc gcc gca cct cta atg gtc gcg ggc aac aca ctc acc 384
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
115 120 125

atg caa tca cag gcc ccg cta acc gtg cac gac tcc aaa ctt agc att 432
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
130 135 140

gcc acc caa gga ccc ctc aca gtg tca gaa gga aag cta gcc ctg caa 480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145 150 155 160

aca tca ggc ccc ctc acc acc acc gat agc agt acc ctt act atc act 528
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165 170 175

gcc tca ccc cct cta act act gcc act ggt agc ttg ggc att gac ttg 576
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190

aaa gag ccc att tat aca caa aat gga aaa cta gga cta aag tac ggg 624
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205

gct cct ttg cat gta aca gac gac cta aac act ttg acc gta gca act 672
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220

ggt cca ggt gtg act att aat aat act tcc ttg caa act aaa gtt act 720
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240

gga gcc ttg ggt ttt gat tca caa ggc aat atg caa ctt aat gta gca 768
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

gga gga cta agg att gat tct caa aac aga cgc ctt ata ctt gat gtt 816
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270

agt tat ccg ttt gat gct caa aac caa cta aat cta aga cta gga cag 864
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285

ggc cct ctt ttt ata aac tca gcc cac aac ttg gat att aac tac aac 912
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290 295 300

aaa ggc ctt tac ttg ttt aca gct tca aac aat tcc aaa aag ctt gag 960
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320

gtt aac cta agc act gcc aag ggg ttg atg ttt gac gct aca gcc ata
1008
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325 330 335

gcc att aat gca gga gat ggg ctt gaa ttt ggt tca cct aat gca cca
1056
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340 345 350

aac aca aat ccc ctc aaa aca aaa att ggc cat ggc cta gaa ttt gat
1104
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365

tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac
1152
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370 375 380

agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act
1200
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385 390 395 400

ttg tgg acc aca cca gct cca gag gct aac tgt aga cta aat gca gag
1248
Leu Trp Thr Thr Pro Ala Pro Glu Ala Asn Cys Arg Leu Asn Ala Glu
405 410 415

aaa gat gct aaa ctc act ttg gtc tta aca aaa tgt ggc agt caa ata
1296
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
420 425 430

ctt gct aca gtt tca gtt ttg gcc ggc agt ttg gct cca ata tct gga
1344
Leu Ala Thr Val Ser Val Leu Ala Gly Ser Leu Ala Pro Ile Ser Gly
435 440 445

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aca gtt caa agt gct cat ctt att ata aga ttt gac gaa aat gga gtg
  1392
Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn Gly Val

    450                 455                 460

cta cta aac aat tcc ttc ctg gac cca gaa tat tgg aac ttt aga aat
  1440
Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe Arg Asn

465                 470                 475                 480

gga gat ctt act gaa ggc aca gcc tat aca aac gct gtt gga ttt atg
  1488
Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe Met

                485                 490                 495

cct aac cta tca gct tat cca aaa tct cac ggt aaa act gcc aaa agt
  1536
Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala Lys Ser

            500                 505                 510

aac att gtc agt caa gtt tac tta aac gga gac aaa act aaa cct gta
  1584
Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys Pro Val

        515                 520                 525

aca cta acc att aca cta aac ggt aca cag gaa aca gga gac aca act
  1632
Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp Thr Thr

    530                 535                 540

cca agt gca tac tct atg tca ttt tca tgg gac tgg tct ggc cac aac
  1680
Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn

545                 550                 555                 560

tac att aat gaa ata ttt gcc aca tcc tct tac act ttt tca tac att
  1728
Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile

                565                 570                 575
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gcc caa gaa taa
1740
Ala Gln Glu

<210> 8
<211> 579
<212> PRT
<213> Artificial

<400> 8

Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100 105 110

Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
115 120 125

Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
130 135 140

Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145 150 155 160

Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr

165 170 175

Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190

Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205

Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220

Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240

Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270

Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290 295 300

Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320

Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325 330 335

Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340 345 350

Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365

Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370 375 380

Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr

385 390 395 400

Leu Trp Thr Thr Pro Ala Pro Glu Ala Asn Cys Arg Leu Asn Ala Glu
405 410 415

Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
420 425 430

Leu Ala Thr Val Ser Val Leu Ala Gly Ser Leu Ala Pro Ile Ser Gly
435 440 445

Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn Gly Val
450 455 460

Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe Arg Asn
465 470 475 480

Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe Met
485 490 495

Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala Lys Ser
500 505 510

Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys Pro Val
515 520 525

Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp Thr Thr
530 535 540

Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn
545 550 555 560

Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile
565 570 575

Ala Gln Glu

<210> 9
<211> 1743
<212> DNA
<213> Artificial

<220>
<223> Codes for a mutated Human Adenovirus type 5 fiber protein. Nucle
otides corresponding to amino acid 441(V) of wild-type fibe
r was
deleted

<220>
<221> CDS
<222> (1)..(1743)

<400> 9
atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca 48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc 96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30

ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct 144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc 192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc 240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac 288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta 336

Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu

100 105 110

act gtg gct gcc gcc gca cct cta atg gtc gcg ggc aac aca ctc acc
384
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr

115 120 125

atg caa tca cag gcc ccg cta acc gtg cac gac tcc aaa ctt agc att
432
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile

130 135 140

gcc acc caa gga ccc ctc aca gtg tca gaa gga aag cta gcc ctg caa
480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln

145 150 155 160

aca tca ggc ccc ctc acc acc acc gat agc agt acc ctt act atc act
528
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr

165 170 175

gcc tca ccc cct cta act act gcc act ggt agc ttg ggc att gac ttg
576
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu

180 185 190

aaa gag ccc att tat aca caa aat gga aaa cta gga cta aag tac ggg
624
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly

195 200 205

gct cct ttg cat gta aca gac gac cta aac act ttg acc gta gca act
672
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr

210 215 220

ggt cca ggt gtg act att aat aat act tcc ttg caa act aaa gtt act
720

Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr

225 230 235 240

gga gcc ttg ggt ttt gat tca caa ggc aat atg caa ctt aat gta gca
768
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala

245 250 255

gga gga cta agg att gat tct caa aac aga cgc ctt ata ctt gat gtt
816
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val

260 265 270

agt tat ccg ttt gat gct caa aac caa cta aat cta aga cta gga cag
864
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln

275 280 285

ggc cct ctt ttt ata aac tca gcc cac aac ttg gat att aac tac aac
912
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn

290 295 300

aaa ggc ctt tac ttg ttt aca gct tca aac aat tcc aaa aag ctt gag
960
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu

305 310 315 320

gtt aac cta agc act gcc aag ggg ttg atg ttt gac gct aca gcc ata
1008
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile

325 330 335

gcc att aat gca gga gat ggg ctt gaa ttt ggt tca cct aat gca cca
1056
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro

340 345 350

aac aca aat ccc ctc aaa aca aaa att ggc cat ggc cta gaa ttt gat
1104

Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365

tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac 1152
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370 375 380

agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act 1200
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385 390 395 400

ttg tgg acc aca cca gct cca tct cct aac tgt aga cta aat gca gag 1248
Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
405 410 415

aaa gat gct aaa ctc act ttg gtc tta aca aaa tgt ggc agt caa ata 1296
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
420 425 430

ctt gct aca gtt tca gtt ttg gct aaa ggc agt ttg gct cca ata tct 1344
Leu Ala Thr Val Ser Val Leu Ala Lys Gly Ser Leu Ala Pro Ile Ser
435 440 445

gga aca gtt caa agt gct cat ctt att ata aga ttt gac gaa aat gga 1392
Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn Gly
450 455 460

gtg cta cta aac aat tcc ttc ctg gac cca gaa tat tgg aac ttt aga 1440
Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe Arg
465 470 475 480

aat gga gat ctt act gaa ggc aca gcc tat aca aac gct gtt gga ttt 1488

Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe
485 490 495

atg cct aac cta tca gct tat cca aaa tct cac ggt aaa act gcc aaa
1536
Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala Lys
500 505 510

agt aac att gtc agt caa gtt tac tta aac gga gac aaa act aaa cct
1584
Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys Pro
515 520 525

gta aca cta acc att aca cta aac ggt aca cag gaa aca gga gac aca
1632
Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp Thr
530 535 540

act cca agt gca tac tct atg tca ttt tca tgg gac tgg tct ggc cac
1680
Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His
545 550 555 560

aac tac att aat gaa ata ttt gcc aca tcc tct tac act ttt tca tac
1728
Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr
565 570 575

att gcc caa gaa taa
1743
Ile Ala Gln Glu
580

<210> 10
<211> 580
<212> PRT
<213> Artificial

<400> 10

Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro

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1               5                   10                  15

Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
            20                  25                  30

Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
        35                  40                  45

Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
    50                  55                  60

Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65                  70                  75                  80

Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
                85                  90                  95

Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
            100                 105                 110

Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
        115                 120                 125

Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
    130                 135                 140

Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145                 150                 155                 160

Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
                165                 170                 175

Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
            180                 185                 190

Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
        195                 200                 205

Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
    210                 215                 220

Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
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225 230 235 240

Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270

Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290 295 300

Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320

Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325 330 335

Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340 345 350

Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365

Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370 375 380

Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385 390 395 400

Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
405 410 415

Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
420 425 430

Leu Ala Thr Val Ser Val Leu Ala Lys Gly Ser Leu Ala Pro Ile Ser
435 440 445

Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn Gly

450 455 460

Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe Arg
465 470 475 480

Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe
485 490 495

Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala Lys
500 505 510

Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys Pro
515 520 525

Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp Thr
530 535 540

Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His
545 550 555 560

Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr
565 570 575

Ile Ala Gln Glu
580

<210> 11
<211> 1743
<212> DNA
<213> Artificial

<220>
<223> Codes for a mutated Human Adenovirus type 5 fiber protein. Nucle
otides corresponding to amino acid 442(K) of wild-type fibe
r was
deleted

<220>
<221> CDS
<222> (1)..(1743)

<400> 11
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Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro

1 5 10 15

tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc
96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro

20 25 30

ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct
144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser

35 40 45

ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc
192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu

50 55 60

aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc
240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser

65 70 75 80

caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac
288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn

85 90 95

ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta
336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu

100 105 110

act gtg gct gcc gcc gca cct cta atg gtc gcg ggc aac aca ctc acc
384
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr

115 120 125

atg caa tca cag gcc ccg cta acc gtg cac gac tcc aaa ctt agc att
432
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile

130 135 140

gcc acc caa gga ccc ctc aca gtg tca gaa gga aag cta gcc ctg caa 480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145 150 155 160

aca tca ggc ccc ctc acc acc acc gat agc agt acc ctt act atc act 528
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165 170 175

gcc tca ccc cct cta act act gcc act ggt agc ttg ggc att gac ttg 576
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190

aaa gag ccc att tat aca caa aat gga aaa cta gga cta aag tac ggg 624
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205

gct cct ttg cat gta aca gac gac cta aac act ttg acc gta gca act 672
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220

ggt cca ggt gtg act att aat aat act tcc ttg caa act aaa gtt act 720
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240

gga gcc ttg ggt ttt gat tca caa ggc aat atg caa ctt aat gta gca 768
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

gga gga cta agg att gat tct caa aac aga cgc ctt ata ctt gat gtt 816
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val

260 265 270

agt tat ccg ttt gat gct caa aac caa cta aat cta aga cta gga cag
864
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln

275 280 285

ggc cct ctt ttt ata aac tca gcc cac aac ttg gat att aac tac aac
912
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn

290 295 300

aaa ggc ctt tac ttg ttt aca gct tca aac aat tcc aaa aag ctt gag
960
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu

305 310 315 320

gtt aac cta agc act gcc aag ggg ttg atg ttt gac gct aca gcc ata
1008
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile

325 330 335

gcc att aat gca gga gat ggg ctt gaa ttt ggt tca cct aat gca cca
1056
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro

340 345 350

aac aca aat ccc ctc aaa aca aaa att ggc cat ggc cta gaa ttt gat
1104
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp

355 360 365

tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac
1152
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp

370 375 380

agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act
1200
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr

385 390 395 400

ttg tgg acc aca cca gct cca tct cct aac tgt aga cta aat gca gag 1248
Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
405 410 415

aaa gat gct aaa ctc act ttg gtc tta aca aaa tgt ggc agt caa ata 1296
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
420 425 430

ctt gct aca gtt tca gtt ttg gct gtt ggc agt ttg gct cca ata tct 1344
Leu Ala Thr Val Ser Val Leu Ala Val Gly Ser Leu Ala Pro Ile Ser
435 440 445

gga aca gtt caa agt gct cat ctt att ata aga ttt gac gaa aat gga 1392
Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn Gly
450 455 460

gtg cta cta aac aat tcc ttc ctg gac cca gaa tat tgg aac ttt aga 1440
Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe Arg
465 470 475 480

aat gga gat ctt act gaa ggc aca gcc tat aca aac gct gtt gga ttt 1488
Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe
485 490 495

atg cct aac cta tca gct tat cca aaa tct cac ggt aaa act gcc aaa 1536
Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala Lys
500 505 510

agt aac att gtc agt caa gtt tac tta aac gga gac aaa act aaa cct 1584
Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys Pro

515 520 525

gta aca cta acc att aca cta aac ggt aca cag gaa aca gga gac aca 1632
Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp Thr
530 535 540

act cca agt gca tac tct atg tca ttt tca tgg gac tgg tct ggc cac 1680
Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His
545 550 555 560

aac tac att aat gaa ata ttt gcc aca tcc tct tac act ttt tca tac 1728
Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr
565 570 575

att gcc caa gaa taa 1743
Ile Ala Gln Glu
580

<210> 12
<211> 580
<212> PRT
<213> Artificial

<400> 12

Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser

65 70 75 80

Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100 105 110

Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
115 120 125

Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
130 135 140

Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145 150 155 160

Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165 170 175

Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190

Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205

Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220

Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240

Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270

Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn

290 295 300

Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320

Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325 330 335

Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340 345 350

Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365

Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370 375 380

Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385 390 395 400

Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
405 410 415

Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
420 425 430

Leu Ala Thr Val Ser Val Leu Ala Val Gly Ser Leu Ala Pro Ile Ser
435 440 445

Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn Gly
450 455 460

Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe Arg
465 470 475 480

Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe
485 490 495

Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala Lys
500 505 510

Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys Pro

515 520 525

Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp Thr
530 535 540

Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His
545 550 555 560

Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr
565 570 575

Ile Ala Gln Glu
580

<210> 13
<211> 1746
<212> DNA
<213> Artificial

<220>
<223> Codes for a mutated Human Adenovirus type 5 fiber protein.

<220>
<221> CDS
<222> (1)..(1746)

<220>
<221> mutation
<222> (1321)..(1326)

<400> 13
atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca
48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro

1 5 10 15

tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc
96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro

20 25 30

ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct
144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser

35 40 45

ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc 192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc 240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac 288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta 336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100 105 110

act gtg gct gcc gcc gca cct cta atg gtc gcg ggc aac aca ctc acc 384
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
115 120 125

atg caa tca cag gcc ccg cta acc gtg cac gac tcc aaa ctt agc att 432
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
130 135 140

gcc acc caa gga ccc ctc aca gtg tca gaa gga aag cta gcc ctg caa 480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145 150 155 160

aca tca ggc ccc ctc acc acc acc gat agc agt acc ctt act atc act 528
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165 170 175

gcc tca ccc cct cta act act gcc act ggt agc ttg ggc att gac ttg 576
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190

aaa gag ccc att tat aca caa aat gga aaa cta gga cta aag tac ggg 624
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205

gct cct ttg cat gta aca gac gac cta aac act ttg acc gta gca act 672
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220

ggt cca ggt gtg act att aat aat act tcc ttg caa act aaa gtt act 720
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240

gga gcc ttg ggt ttt gat tca caa ggc aat atg caa ctt aat gta gca 768
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

gga gga cta agg att gat tct caa aac aga cgc ctt ata ctt gat gtt 816
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270

agt tat ccg ttt gat gct caa aac caa cta aat cta aga cta gga cag 864
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285

ggc cct ctt ttt ata aac tca gcc cac aac ttg gat att aac tac aac 912
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290 295 300

aaa ggc ctt tac ttg ttt aca gct tca aac aat tcc aaa aag ctt gag 960
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320

gtt aac cta agc act gcc aag ggg ttg atg ttt gac gct aca gcc ata 1008
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325 330 335

gcc att aat gca gga gat ggg ctt gaa ttt ggt tca cct aat gca cca 1056
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340 345 350

aac aca aat ccc ctc aaa aca aaa att ggc cat ggc cta gaa ttt gat 1104
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365

tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac 1152
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370 375 380

agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act 1200
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385 390 395 400

ttg tgg acc aca cca gct cca tct cct aac tgt aga cta aat gca gag 1248
Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
405 410 415

aaa gat gct aaa ctc act ttg gtc tta aca aaa tgt ggc agt caa ata 1296
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
420 425 430

ctt gct aca gtt tca gtt ttg gct gct gca ggc agt ttg gct cca ata
1344
Leu Ala Thr Val Ser Val Leu Ala Ala Ala Gly Ser Leu Ala Pro Ile
435 440 445

tct gga aca gtt caa agt gct cat ctt att ata aga ttt gac gaa aat
1392
Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
450 455 460

gga gtg cta cta aac aat tcc ttc ctg gac cca gaa tat tgg aac ttt
1440
Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
465 470 475 480

aga aat gga gat ctt act gaa ggc aca gcc tat aca aac gct gtt gga
1488
Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
485 490 495

ttt atg cct aac cta tca gct tat cca aaa tct cac ggt aaa act gcc
1536
Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
500 505 510

aaa agt aac att gtc agt caa gtt tac tta aac gga gac aaa act aaa
1584
Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
515 520 525

cct gta aca cta acc att aca cta aac ggt aca cag gaa aca gga gac
1632
Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp
530 535 540

aca act cca agt gca tac tct atg tca ttt tca tgg gac tgg tct ggc
1680
Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly
545 550 555 560

cac aac tac att aat gaa ata ttt gcc aca tcc tct tac act ttt tca
1728
His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser
565 570 575

tac att gcc caa gaa taa
1746
Tyr Ile Ala Gln Glu
580

<210> 14
<211> 581
<212> PRT
<213> Artificial

<400> 14

Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100 105 110

Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
115 120 125

Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile

130 135 140

Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145 150 155 160

Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165 170 175

Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190

Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205

Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220

Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240

Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270

Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290 295 300

Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320

Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325 330 335

Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340 345 350

Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp

355 360 365

Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370 375 380

Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385 390 395 400

Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
405 410 415

Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
420 425 430

Leu Ala Thr Val Ser Val Leu Ala Ala Ala Gly Ser Leu Ala Pro Ile
435 440 445

Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
450 455 460

Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
465 470 475 480

Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
485 490 495

Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
500 505 510

Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
515 520 525

Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp
530 535 540

Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly
545 550 555 560

His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser
565 570 575

Tyr Ile Ala Gln Glu

580

<210> 15
<211> 1746
<212> DNA
<213> Artificial

<220>
<223> Codes for a mutated Human Adenovirus type 5 fiber protein.

<220>
<221> CDS
<222> (1)..(1746)

<220>
<221> mutation
<222> (1378)..(1380)

<400> 15
atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca 48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc 96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30

ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct 144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc 192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc 240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac

288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn

85 90 95

ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta
336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu

100 105 110

act gtg gct gcc gcc gca cct cta atg gtc gcg ggc aac aca ctc acc
384
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr

115 120 125

atg caa tca cag gcc ccg cta acc gtg cac gac tcc aaa ctt agc att
432
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile

130 135 140

gcc acc caa gga ccc ctc aca gtg tca gaa gga aag cta gcc ctg caa
480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln

145 150 155 160

aca tca ggc ccc ctc acc acc acc gat agc agt acc ctt act atc act
528
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr

165 170 175

gcc tca ccc cct cta act act gcc act ggt agc ttg ggc att gac ttg
576
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu

180 185 190

aaa gag ccc att tat aca caa aat gga aaa cta gga cta aag tac ggg
624
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly

195 200 205

gct cct ttg cat gta aca gac gac cta aac act ttg acc gta gca act

672
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr

210 215 220

ggt cca ggt gtg act att aat aat act tcc ttg caa act aaa gtt act
720
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr

225 230 235 240

gga gcc ttg ggt ttt gat tca caa ggc aat atg caa ctt aat gta gca
768
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala

245 250 255

gga gga cta agg att gat tct caa aac aga cgc ctt ata ctt gat gtt
816
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val

260 265 270

agt tat ccg ttt gat gct caa aac caa cta aat cta aga cta gga cag
864
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln

275 280 285

ggc cct ctt ttt ata aac tca gcc cac aac ttg gat att aac tac aac
912
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn

290 295 300

aaa ggc ctt tac ttg ttt aca gct tca aac aat tcc aaa aag ctt gag
960
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu

305 310 315 320

gtt aac cta agc act gcc aag ggg ttg atg ttt gac gct aca gcc ata
1008
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile

325 330 335

gcc att aat gca gga gat ggg ctt gaa ttt ggt tca cct aat gca cca

1056
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro

340 345 350

aac aca aat ccc ctc aaa aca aaa att ggc cat ggc cta gaa ttt gat
1104
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp

355 360 365

tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac
1152
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp

370 375 380

agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act
1200
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr

385 390 395 400

ttg tgg acc aca cca gct cca tct cct aac tgt aga cta aat gca gag
1248
Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu

405 410 415

aaa gat gct aaa ctc act ttg gtc tta aca aaa tgt ggc agt caa ata
1296
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile

420 425 430

ctt gct aca gtt tca gtt ttg gct gtt aaa ggc agt ttg gct cca ata
1344
Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile

435 440 445

tct gga aca gtt caa agt gct cat ctt att ata gaa ttc gac gaa aat
1392
Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Glu Phe Asp Glu Asn

450 455 460

gga gtg cta cta aac aat tcc ttc ctg gac cca gaa tat tgg aac ttt

1440
Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe

465 470 475 480

aga aat gga gat ctt act gaa ggc aca gcc tat aca aac gct gtt gga
1488
Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly

485 490 495

ttt atg cct aac cta tca gct tat cca aaa tct cac ggt aaa act gcc
1536
Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala

500 505 510

aaa agt aac att gtc agt caa gtt tac tta aac gga gac aaa act aaa
1584
Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys

515 520 525

cct gta aca cta acc att aca cta aac ggt aca cag gaa aca gga gac
1632
Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp

530 535 540

aca act cca agt gca tac tct atg tca ttt tca tgg gac tgg tct ggc
1680
Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly

545 550 555 560

cac aac tac att aat gaa ata ttt gcc aca tcc tct tac act ttt tca
1728
His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser

565 570 575

tac att gcc caa gaa taa
1746
Tyr Ile Ala Gln Glu

580

<210> 16
<211> 581
<212> PRT
<213> Artificial

<400> 16

Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100 105 110

Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
115 120 125

Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
130 135 140

Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145 150 155 160

Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165 170 175

Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190

Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly

195 200 205

Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220

Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240

Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270

Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290 295 300

Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320

Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325 330 335

Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340 345 350

Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365

Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370 375 380

Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385 390 395 400

Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
405 410 415

Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile

420 425 430

Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
435 440 445

Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Glu Phe Asp Glu Asn
450 455 460

Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
465 470 475 480

Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
485 490 495

Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
500 505 510

Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
515 520 525

Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp
530 535 540

Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly
545 550 555 560

His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser
565 570 575

Tyr Ile Ala Gln Glu
580

<210> 17
<211> 1740
<212> DNA
<213> Artificial

<220>
<223> Codes for a mutated Human Adenovirus type 5 fiber protein. Nucle
otides corresponding to amino acids 509(G) & 510(K) of wild -type
fiber were deleted

<220>

<221> CDS
<222> (1)..(1740)

<400> 17
atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca 48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc 96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30

ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct 144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc 192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc 240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac 288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta 336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100 105 110

act gtg gct gcc gcc gca cct cta atg gtc gcg ggc aac aca ctc acc 384
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr

115 120 125

atg caa tca cag gcc ccg cta acc gtg cac gac tcc aaa ctt agc att
432
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile

130 135 140

gcc acc caa gga ccc ctc aca gtg tca gaa gga aag cta gcc ctg caa
480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln

145 150 155 160

aca tca ggc ccc ctc acc acc acc gat agc agt acc ctt act atc act
528
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr

165 170 175

gcc tca ccc cct cta act act gcc act ggt agc ttg ggc att gac ttg
576
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu

180 185 190

aaa gag ccc att tat aca caa aat gga aaa cta gga cta aag tac ggg
624
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly

195 200 205

gct cct ttg cat gta aca gac gac cta aac act ttg acc gta gca act
672
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr

210 215 220

ggt cca ggt gtg act att aat aat act tcc ttg caa act aaa gtt act
720
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr

225 230 235 240

gga gcc ttg ggt ttt gat tca caa ggc aat atg caa ctt aat gta gca
768
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala

245 250 255

gga gga cta agg att gat tct caa aac aga cgc ctt ata ctt gat gtt 816
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270

agt tat ccg ttt gat gct caa aac caa cta aat cta aga cta gga cag 864
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285

ggc cct ctt ttt ata aac tca gcc cac aac ttg gat att aac tac aac 912
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290 295 300

aaa ggc ctt tac ttg ttt aca gct tca aac aat tcc aaa aag ctt gag 960
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320

gtt aac cta agc act gcc aag ggg ttg atg ttt gac gct aca gcc ata 1008
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325 330 335

gcc att aat gca gga gat ggg ctt gaa ttt ggt tca cct aat gca cca 1056
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340 345 350

aac aca aat ccc ctc aaa aca aaa att ggc cat ggc cta gaa ttt gat 1104
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365

tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac 1152
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp

370 375 380

agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act
1200
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr

385 390 395 400

ttg tgg acc aca cca gct cca tct cct aac tgt aga cta aat gca gag
1248
Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu

405 410 415

aaa gat gct aaa ctc act ttg gtc tta aca aaa tgt ggc agt caa ata
1296
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile

420 425 430

ctt gct aca gtt tca gtt ttg gct gtt aaa ggc agt ttg gct cca ata
1344
Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile

435 440 445

tct gga aca gtt caa agt gct cat ctt att ata aga ttt gac gaa aat
1392
Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn

450 455 460

gga gtg cta cta aac aat tcc ttc ctg gac cca gaa tat tgg aac ttt
1440
Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe

465 470 475 480

aga aat gga gat ctt act gaa ggc aca gcc tat aca aac gct gtt gga
1488
Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly

485 490 495

ttt atg cct aac cta tca gct tat cca aaa tct cac act gcc aaa agt
1536
Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Thr Ala Lys Ser

500 505 510

aac att gtc agt caa gtt tac tta aac gga gac aaa act aaa cct gta 1584
Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys Pro Val
515 520 525

aca cta acc att aca cta aac ggt aca cag gaa aca gga gac aca act 1632
Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp Thr Thr
530 535 540

cca agt gca tac tct atg tca ttt tca tgg gac tgg tct ggc cac aac 1680
Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn
545 550 555 560

tac att aat gaa ata ttt gcc aca tcc tct tac act ttt tca tac att 1728
Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile
565 570 575

gcc caa gaa taa 1740
Ala Gln Glu

<210> 18
<211> 579
<212> PRT
<213> Artificial

<400> 18

Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser

35 40 45

Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100 105 110

Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
115 120 125

Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
130 135 140

Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145 150 155 160

Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165 170 175

Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190

Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205

Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220

Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240

Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val

260 265 270

Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290 295 300

Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320

Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325 330 335

Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340 345 350

Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365

Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370 375 380

Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385 390 395 400

Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
405 410 415

Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
420 425 430

Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
435 440 445

Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
450 455 460

Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
465 470 475 480

Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly

485 490 495

Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Thr Ala Lys Ser
500 505 510

Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys Pro Val
515 520 525

Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp Thr Thr
530 535 540

Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn
545 550 555 560

Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile
565 570 575

Ala Gln Glu

<210> 19
<211> 1740
<212> DNA
<213> Artificial

<220>
<223> Codes for a mutated Human Adenovirus type 5 fiber protein. Nucle
otides corresponding to amino acids 538(G) & 539(T) of wild -type
fiber were deleted

<220>
<221> CDS
<222> (1)..(1740)

<400> 19
atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca 48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc 96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30

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ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct
   144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser

        35                  40                  45

ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc
   192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu

    50                  55                  60

aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc
   240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser

65                  70                  75                  80

caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac
   288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn

                85                  90                  95

ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta
   336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu

            100                 105                 110

act gtg gct gcc gcc gca cct cta atg gtc gcg ggc aac aca ctc acc
   384
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr

        115                 120                 125

atg caa tca cag gcc ccg cta acc gtg cac gac tcc aaa ctt agc att
   432
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile

    130                 135                 140

gcc acc caa gga ccc ctc aca gtg tca gaa gga aag cta gcc ctg caa
   480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln

145                 150                 155                 160
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aca tca ggc ccc ctc acc acc acc gat agc agt acc ctt act atc act 528
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165 170 175

gcc tca ccc cct cta act act gcc act ggt agc ttg ggc att gac ttg 576
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190

aaa gag ccc att tat aca caa aat gga aaa cta gga cta aag tac ggg 624
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205

gct cct ttg cat gta aca gac gac cta aac act ttg acc gta gca act 672
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220

ggt cca ggt gtg act att aat aat act tcc ttg caa act aaa gtt act 720
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240

gga gcc ttg ggt ttt gat tca caa ggc aat atg caa ctt aat gta gca 768
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

gga gga cta agg att gat tct caa aac aga cgc ctt ata ctt gat gtt 816
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270

agt tat ccg ttt gat gct caa aac caa cta aat cta aga cta gga cag 864
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285

ggc cct ctt ttt ata aac tca gcc cac aac ttg gat att aac tac aac 912
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290 295 300

aaa ggc ctt tac ttg ttt aca gct tca aac aat tcc aaa aag ctt gag 960
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320

gtt aac cta agc act gcc aag ggg ttg atg ttt gac gct aca gcc ata 1008
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325 330 335

gcc att aat gca gga gat ggg ctt gaa ttt ggt tca cct aat gca cca 1056
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340 345 350

aac aca aat ccc ctc aaa aca aaa att ggc cat ggc cta gaa ttt gat 1104
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365

tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac 1152
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370 375 380

agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act 1200
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385 390 395 400

ttg tgg acc aca cca gct cca tct cct aac tgt aga cta aat gca gag 1248
Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
405 410 415

aaa gat gct aaa ctc act ttg gtc tta aca aaa tgt ggc agt caa ata 1296
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
420 425 430

ctt gct aca gtt tca gtt ttg gct gtt aaa ggc agt ttg gct cca ata 1344
Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
435 440 445

tct gga aca gtt caa agt gct cat ctt att ata aga ttt gac gaa aat 1392
Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
450 455 460

gga gtg cta cta aac aat tcc ttc ctg gac cca gaa tat tgg aac ttt 1440
Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
465 470 475 480

aga aat gga gat ctt act gaa ggc aca gcc tat aca aac gct gtt gga 1488
Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
485 490 495

ttt atg cct aac cta tca gct tat cca aaa tct cac ggt aaa act gcc 1536
Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
500 505 510

aaa agt aac att gtc agt caa gtt tac tta aac gga gac aaa act aaa 1584
Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
515 520 525

cct gta aca cta acc att aca cta aac cag gaa aca gga gac aca act 1632
Pro Val Thr Leu Thr Ile Thr Leu Asn Gln Glu Thr Gly Asp Thr Thr
530 535 540

cca agt gca tac tct atg tca ttt tca tgg gac tgg tct ggc cac aac
1680
Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn
545 550 555 560

tac att aat gaa ata ttt gcc aca tcc tct tac act ttt tca tac att
1728
Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile
565 570 575

gcc caa gaa taa
1740
Ala Gln Glu

<210> 20
<211> 579
<212> PRT
<213> Artificial

<400> 20

Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95

Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu

100 105 110

Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
115 120 125

Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
130 135 140

Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145 150 155 160

Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165 170 175

Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190

Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205

Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220

Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240

Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270

Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290 295 300

Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320

Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile

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                    325                 330                 335

Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
            340                 345                 350

Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
        355                 360                 365

Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
    370                 375                 380

Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385                 390                 395                 400

Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
                405                 410                 415

Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
            420                 425                 430

Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
        435                 440                 445

Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
    450                 455                 460

Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
465                 470                 475                 480

Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
                485                 490                 495

Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
            500                 505                 510

Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
        515                 520                 525

Pro Val Thr Leu Thr Ile Thr Leu Asn Gln Glu Thr Gly Asp Thr Thr
    530                 535                 540

Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn
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545 550 555 560

Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile
565 570 575

Ala Gln Glu

<210> 21
<211> 38
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(38)

<400> 21
accacaccag ctccagaggc taactgtaga ctaaatgc
38

<210> 22
<211> 38
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(38)

<400> 22
gcatttagtc tacagttagc ctctggagct ggtgtgtt
38

<210> 23
<211> 38
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(38)

<400> 23
acagtttcag ttttggccgg cagtttggct ccaatatc
38

<210> 24
<211> 38
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(38)

<400> 24
gatattggag ccaaactgcc ggccaaaact gaaactgt
38

<210> 25
<211> 36
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(36)

<400> 25
acagtttcag ttttggctaa aggcagtttg gctcca
36

<210> 26
<211> 36
<212> DNA
<213> Artificial

<220>
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<220>
<221> primer_bind
<222> (1)..(36)

<400> 26
tggagccaaa ctgcctttag ccaaaactga aactgt
36

<210> 27
<211> 36
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(36)

<400> 27
gtttcagttt tggctgttgg cagtttggct ccaata
36

<210> 28
<211> 36
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(36)

<400> 28
tattggagcc aaactgccaa cagccaaaac tgaaac
36

<210> 29
<211> 36
<212> DNA
<213> Artificial

<220>
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<220>
<221> primer_bind
<222> (1)..(36)

<400> 29
gtttcagttt tggctgctgc aggcagtttg gctcca
36

<210> 30
<211> 36
<212> DNA
<213> Artificial

<220>
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<220>
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<222> (1)..(36)

<400> 30
tggagccaaa ctgcctgcag cagccaaaac tgaaac
36

<210> 31
<211> 36
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(36)

<400> 31
gctcatctta ttatagaatt cgacgaaaat ggagtg
36

<210> 32
<211> 36
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(36)

<400> 32
cactccattt tcgtcgaatt ctataataag atgagc
36

<210> 33
<211> 39
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>

<221> primer_bind
<222> (1)..(39)

<400> 33
gcttatccaa aatctcacac tgccaaaagt aacattgtc
39

<210> 34
<211> 39
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(39)

<400> 34
gacaatgtta cttttggcag tgtgagattt tggataagc
39

<210> 35
<211> 35
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(35)

<400> 35
ctaaccatta cactaaacca ggaaacagga gacac
35

<210> 36
<211> 35
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<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(35)

<400> 36
gtgtctcctg tttcctggtt tagtgtaatg gttag

35

<210> 37
<211> 33
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(33)

<400> 37
ataagatttg acgaaactgg agtgctacta aac
33

<210> 38
<211> 33
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(33)

<400> 38
gtttagtagc actccagttt cgtcaaatct tat
33

<210> 39
<211> 33
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
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<222> (1)..(33)

<400> 39
tttgacgaaa atggacacct actaaacaat tcc
33

<210> 40
<211> 33

<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(33)

<400> 40
ggaattgttt agtaggtgtc cagtttcgtc aaa
33

<210> 41
<211> 33
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(33)

<400> 41
aacctatcag cttatgcaaa atctcacggt aaa
33

<210> 42
<211> 32
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<220>
<221> primer_bind
<222> (1)..(32)

<400> 42
tttaccgtga gattttgcat aagctgatag gt
32

<210> 43
<211> 10
<212> PRT
<213> Artificial

<220>
<223> cRGD consensus sequence

<400> 43

His Cys Asp Cys Arg Gly Asp Cys Phe Cys
1 5 10